

REMARKS

Claims 23-26 have been amended and are pending. In compliance with 37 C.F.R. § 1.121(c), Applicants enclose a marked up version of the amended claims, showing all of the changes relative to the previous claim versions. In compliance with 37 C.F.R. § 1.121(b), Applicants enclose marked up versions of amended pages 1, 13, 17 and 19, showing the relative changes.

Applicants acknowledge that Examiner Devi spoke to Dr. Bernhard D. Saxe, the attorney of record, on January 22, 2001, concerning descriptive support for the peptide claimed in claim 24. Dr. Saxe indicated that Applicants would address this issue upon receipt of an Official Action.

I. REJECTIONS UNDER 35 U.S.C. §112, ¶2

The examiner rejects claims 23-26 under 35 U.S.C. §112, ¶1, for alleged indefiniteness. The Examiner asserts that the claims are confusing because of the recitation of the term "SEQ ID NO:1." Applicants assert that the proposed amendments obviate these rejections.

II. REJECTIONS UNDER 35 U.S.C. §112, ¶1

The examiner rejects claim 24 under 35 U.S.C. §112, ¶1 for allegedly failing to convey to one of skill in the art that the Applicants possessed the claimed invention at the time the application was filed. In support of this rejection, the examiner asserts that there is no written support in the original specification for an isolated peptide or polypeptide consisting of an amino acid sequence from position 27 to position 229 of SEQ ID NO: 2. Applicants respectfully traverse this rejection.

In levying a written description rejection, an examiner has the burden of presenting by a preponderance of the evidence why a person skilled in the art would not recognize in an applicant's disclosure, a description of the invention defined by the claims. *See In re Wertheim*, 541 F.2d 257, 263 (CCPA 1976). Applicants assert that the Examiner has failed to meet her burden.

The fundamental factual inquiry regarding the adequacy of disclosure is whether the application conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the claimed invention. *See Vas-Cath, Inc. v.*

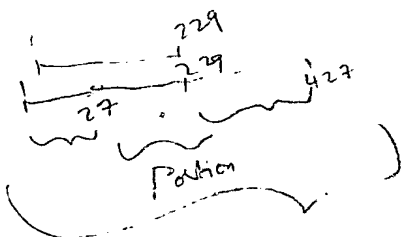
Mahurkar, 935 F.2d 1555, 1563 (Fed. Cir. 1991). To provide descriptive support, it is not necessary that the application describe the claim limitations exactly. *See e.g. In re Lukach*, 442 F.2d 967, 969 (CCPA 1971)([T]he invention claimed does not have to be described *in ipso verbis* in order to satisfy the description requirement of § 112.) Rather, the application need only be sufficiently clear that persons of skill in the art would recognize that the Applicant had possession of the claimed invention. *See In re Wertheim*, 541 F.2d at 263. Thus, the written description requirement is satisfied when each claim limitation is supported explicitly, implicitly or inherently in the originally filed disclosure. *See Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112 ¶1*, "Written Description" Requirement, 66 Fed. Reg. 1099 (2001).

In the instant case, the specification discloses that a peptide consisting of amino acids 1 to 229 of the peptide described in figure 2 (*i.e.* SEQ ID NO:2) is advantageous for preparing antibodies which specifically recognize human interferon class I receptor (IFN-R). *See* Application page 8, lines 22-27. The application further discloses an antibody specific for an epitope on the peptide consisting of amino acids 27 to 427 of the peptide described in figure 2. *See* Application page 10, lines 11-16 and original claims 9 and 12.

Applicants assert that an artisan reading the disclosure would recognize readily that Applicants had determined, by the time the application was filed, that amino acids 1 to 26 and 230 to 427 of SEQ ID NO:2 were not essential for the proper folding of the extracellular domain of the IFN-R encoded by amino acids 27 to 427. Thus, one of ordinary skill in the art would recognize that Applicants had possession of a peptide consisting of an amino acid sequence from position 27 to position 229 of SEQ ID NO:2, wherein the peptide or portion thereof specifically binds to monoclonal antibody 64G12.

Accordingly, Applicants assert that the written description requirement is satisfied. Therefore, withdrawal of these rejections is requested.

The examiner also rejects claims 23-26 under 35 U.S.C. §112, ¶1 for allegedly failing to enable an artisan to practice the claimed invention. The Examiner asserts that since SEQ ID NO:1 refers to nucleic acid, and not a peptide, the scope of the claimed invention differs from that of the disclosure. Applicants believe the proposed amendments obviate these rejections.



III. REJECTIONS UNDER 35 U.S.C. §102

The examiner rejects claims 23-25 under 35 U.S.C. §102(a) for allegedly being anticipated by Racaniello *et al.* Applicants respectfully traverse this rejection.

The examiner asserts that "the prior art peptide or polypeptide is viewed as the same as the Applicant's peptide or polypeptide." This assertion is erroneous as a matter of law, however.

The instant claims are directed to an isolated peptide or polypeptide *consisting of* an amino acid sequence from position 27 to position 229 of SEQ ID NO: 2 or a portion thereof. The transitional phrase "consisting of" excludes any element, step or ingredient not specified in the claim. *See* MPEP §2111.03 (August 2001)(referencing *In re Gray*, 53 F.2d 520 (CCPA 1931)). Thus, a peptide of the claimed invention can be only an amino acid sequence from position 27 to position 229 of SEQ ID NO: 2 or a portion thereof.

In contrast, the seven amino acid "peptide" identified by the Examiner is in fact a portion of a much larger peptide, which comprises 2206 amino acids. Since the prior art peptide contains more elements than those recited in the instant claim, it can not, as a matter of law, be viewed as equivalent to the Applicant's peptide. Therefore, the prior art peptide cannot anticipate the claimed invention. Accordingly, Applicants assert that this rejection is erroneous as a matter of law, and respectfully request that it be withdrawn.

IV. OBJECTIONS

The examiner objects to claim 25 for capitalizing the conjunction "OR." Applicants believe the proposed amendment obviates the objection.

The examiner objects to claims 25-26 for not identifying the 64G12 monoclonal antibody by its depository accession number. Applicants believe the proposed amendments obviate these objections.

The Examiner has maintained her objection to the drawings. Applicants submit herein replacement drawings, which they believe obviate these objections.

The Examiner asserts that the Declaration is defective because the inventors made handwritten corrections to their countries of citizenship or name-spelling. In compliance with 37 C.F.R. § 1.67, Applicants herein provide a substitute Declaration. After a diligent effort, however, not all of the inventors' signatures were obtained. In accordance with MPEP § 409.03(d), Julia Andral-Ziurys, Applicant's patent counsel, made a diligent effort

Amendments
2/20/03
41

to contact all of the inventors. Substitute declarations were forwarded, up to three times over five months, to the last known address of each inventor. To date, Applicants have not received signed declarations from Patrick Benoit or Deborah Maquire.

In further compliance with MPEP § 409.03(d), Ms. Andral-Ziurys has prepared a declaration which provides a statement of facts describing her efforts to contact the absent inventors. Documents supporting Ms. Andral-Ziurys' efforts are enclosed as Annexes 1-21. In addition, the last known addresses of the absent inventors are provided below:

Patrick Benoit: 24 Rue Jonquoy, F-75014 Paris, France

Deborah Maquire: 24, rue Maitre-Albert, F-75005 Paris, France

Accordingly, as the burden for filing a substitute declaration has been satisfied, Applicants believe the objection has been obviated.

The Examiner objects to the title. Applicants believe the proposed amendment obviates the objection.

The Examiner objects to the recitation in the claims of the term "SEQ ID NO:1." Applicants believe the proposed amendments obviate the objection.

The Examiner objects to the figure legends for Figures 2 and 3. Applicants believe the proposed amendments obviate these objections.

The Examiner also objects to the heading used for the figures. Applicants believe the proposed amendment obviates the objection.


The Examiner objects to the use of the terms "Mono Q" and "Tween 20" without designating them as trademarks. Applicants believe the proposed amendments obviate these objections.

In view of the foregoing amendments and remarks it is believed that the application now is in condition for allowance. A favorable disposition of the application therefore is solicited. The examiner also is invited to contact the undersigned if there are any questions or if the examiner believes that further discussion will advance prosecution.

Respectfully submitted,

Dec 7, 2001

Date



Bernhard D. Saxe

Registration No. 28,665

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Washington, D.C. 20007-5109
(202) 672-5300

Marked Up Version of Claim Amendments

Claim 23. (Fourth Amendment) An isolated peptide or polypeptide which is a fragment of the extracellular portion of the interferon receptor (IFN-R) of SEQ ID NO: [1 or] 2, said peptide or polypeptide consisting of an amino acid sequence from position 27 to position 427 of SEQ ID NO: [1 or] 2 or a portion thereof; wherein said peptide or polypeptide or a portion thereof specifically binds to monoclonal antibody 64G12 deposited at the ECACC under accession no. 92022605.

102(c)
583112
5822516
5643749

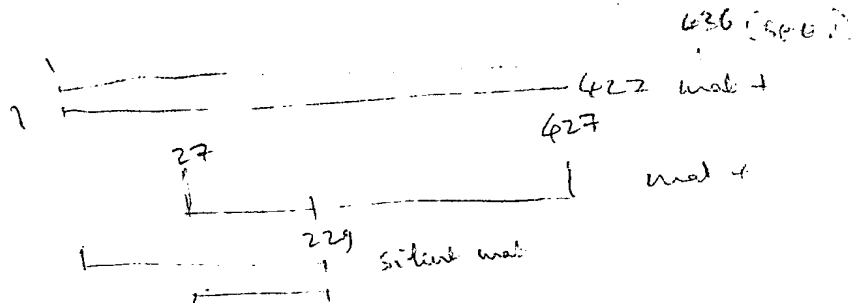
Claim 24. (Fourth Amendment) An isolated peptide or polypeptide consisting of an amino acid sequence from position 27 to position 229 of SEQ ID NO: [1 or] 2 or a portion thereof; wherein said peptide or polypeptide or portion thereof specifically binds to monoclonal antibody 64G12 deposited at the ECACC under accession no. 92022605.

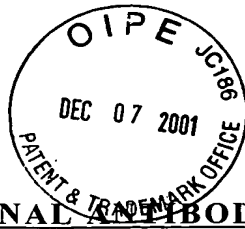
Claim 25. (Fourth Amendment) An isolated peptide or polypeptide which is a fragment of the extracellular portion of the interferon receptor (IFN-R) of SEQ ID NO: [1 or] 2, said peptide or polypeptide consisting of an amino acid sequence from position 1 to position 229 of SEQ ID NO: [1 OR] 2 or a portion thereof; wherein said peptide or polypeptide or a portion thereof specifically binds to monoclonal antibody 64G12 deposited at the ECACC under accession no. 92022605.

Claim 26. (Third Amendment) An isolated peptide or polypeptide which is derived from a peptide or polypeptide as claimed in claim 23 by substitution of one or more amino acid residues and which retains the ability to specifically bind to monoclonal antibody 64G12 deposited at the ECACC under accession no. 92022605.

Should be included.
Broadening.

Table 1 shows that
64G12 is specific
27-427





**[MONOCLONAL ANTIBODIES AGAINST THE
INTERFERON RECEPTOR, WITH NEUTRALIZING
ACTIVITY AGAINST TYPE I INTERFERON] AN ISOLATED PEPTIDE
OR POLYPEPTIDE OF THE EXTRACELLULAR PORTION OF THE
HUMAN INTERFERON RECEPTOR (IFN-R)**

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The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN: alpha (α), beta (β) and gama. IFN α and IFN β together are known as the type I interferon.

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Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33: 251, 1986).

15

The human IFN α locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, α II or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFN α genes (Weissmann and Weber 1986).

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The subtypes of IFN α have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78: 2848, 1981) and have the same cellular receptor (Agnat M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

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The interferon β (IFN β) is encoded by a single gene which has approximately 50% homology with the IFN α genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

[FIGURES] BRIEF DESCRIPTION OF THE DRAWINGS

-- Figure 1: binding of ^{125}I -labelled monoclonal antibodies 34F10 and 64G12 to:

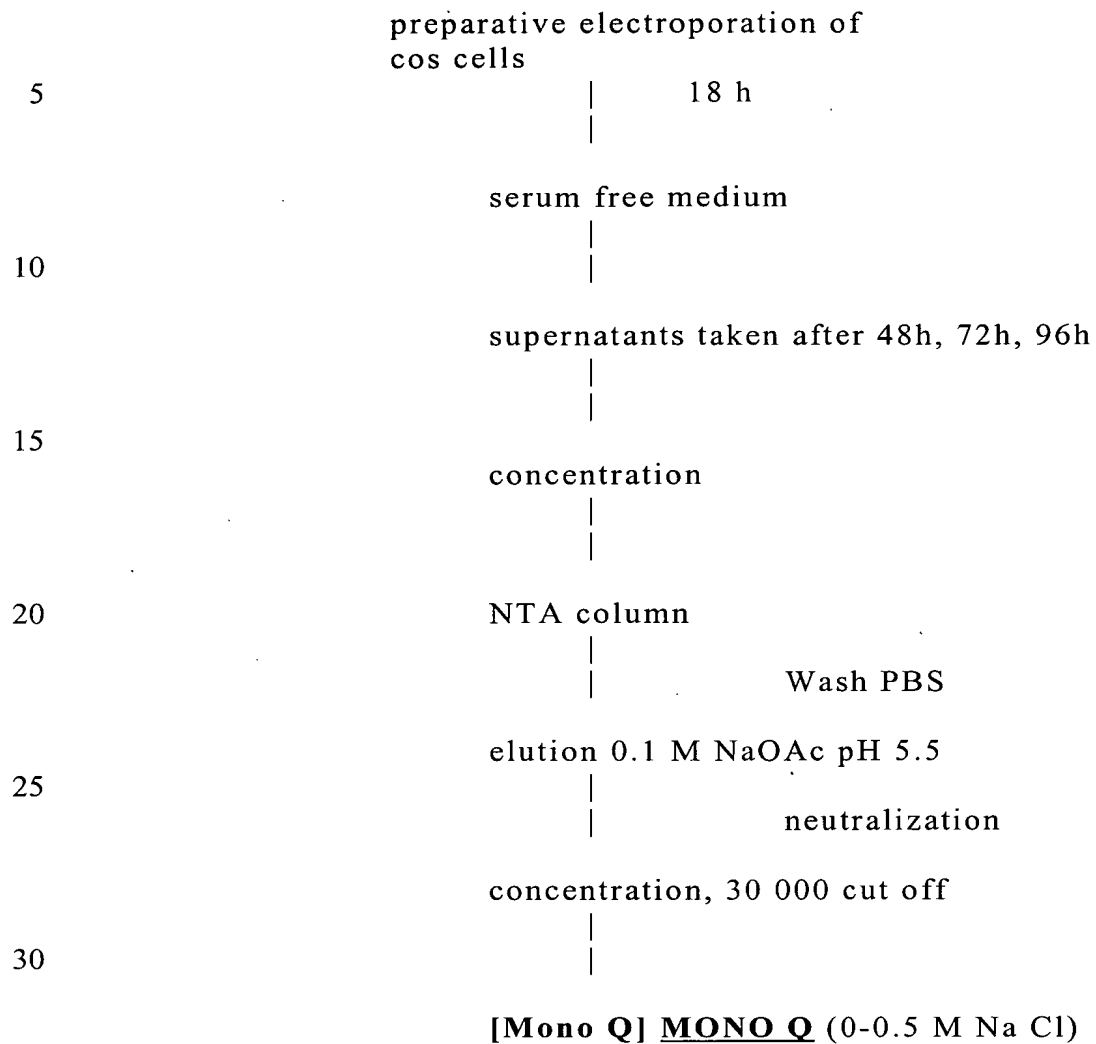
- 5 -- A: Daudi cells
 -- B: Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then
10 washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was measured by incubation with a 100 fold [exces] excess of cold antibodies and [substracted] subtracted from total counts.

-- [Figure 2] Figures 2A and 2B: nucleotide and
15 corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthesized in either procaryotic cells (E.coli) or a mammalian cell
20 system (Cos cell). These soluble forms were based on the DNA sequence described in [figure 2] Figures 2A and 2B.

-- [Figure 3] Figures 3A and 3B: nucleotide and corresponding amino-acid sequence of the human IFN-R.

Purification of the soluble IFN-R from Cos7 cells

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35: 1-32, 1980). Briefly, 5×10^7 spleen cells were fused to 3×10^7 myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as 20×10^7 spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method:

- a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,
- b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C ,
- c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.5% **[Tween 20]** TWEEN 20,
- d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% **[Tween 20]** TWEEN 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

**PATENT**

Attorney Docket No. 017283/0123

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of :
 BENOIT et al. :
 : Group Art Unit: 1641
 Serial No.: 09/240,675 :
 : Examiner: DEVI, S.
 Filed: February 2, 1999

For: MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH
 NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

DECLARATION

Hon. Commissioner of Patents
 and Trademarks
 Washington, D.C. 20231

I, Julia ANDRAL-ZIURYS, do hereby declare:

1. That I am a U.S. Patent Attorney having registration No. 33,823.
2. That I am currently a partner working at Ernest Gutmann - Yves Plasseraud SA, an Industrial Property law firm in Paris, France. One of my responsibilities in my current position is to aid my colleagues with U.S. Patent practice.
3. That on March 5, 2001, a copy of an Official Action sent on January 31, 2001 by the US Patent and Trademark Office concerning the above-identified patent application was received by a fellow partner, namely Anne DESAIX who is responsible for prosecution of this patent application worldwide.
4. Anne DESAIX was working on the prosecution of this U.S. patent application at the time of receipt of the official action in paragraph (3) with a young collaborator in the firm, namely Véronique MARCADÉ.

5. That the Examiner in the U.S. Official Action set forth in paragraph (3) requested that a new oath and Declaration be submitted since the original Declaration was deemed to be defective due to the fact that hand-written changes were made by the inventors without being properly initialed and dated.
6. That on May 31, 2001, a letter was sent to the client (LEB-TECH attention: Mrs. LAGNEAU) and one of the inventors Mr. Tovey, with a copy of a New Declaration and Power of Attorney, by Anne DESAIX and Véronique MARCADÉ (*Annex 1*).
7. On June 21, 2001, Anne DESAIX and Véronique MARCADÉ sent a facsimile to Ms. Lagneau, to remind her of their letter of May 31, 2001, and to suggest that our firm send the declarations to the inventors (*Annex 2*).
8. That sometime after receipt of the U.S. Official Action in paragraph (3), I was approached by my Partner Anne DESAIX requesting that I oversee the handling of the new Declaration according to U.S. practice and that I was to work with Véronique MARCADÉ.
9. On July 6, and July 10, 2001, Véronique MARCADÉ talked on the telephone with Ms. VOULGARIS, working at CFPI Nufarm with Ms. LAGNEAU. Ms. VOULGARIS indicated that she knew the address of three of the inventors (those of Mr. TOVEY, Mr. MEYER, and Mr. BENOIT), but that neither she nor Mr. TOVEY knew the current address of Mr. PLAVEC and Ms. MAGUIRE. This was confirmed by two letters, dated July 11 and 12. Copies of these letters are enclosed, together with their translation into English (*Annex 3*). The letter of July 12, 2001 also contained the telephone number of Mr. PLAVEC, now working in the US.
10. On around July 16, 2001, a registered package was sent to the last known address of each of the inventors. The package included copy of the Declaration of 11/11/94, signed by the inventors, a copy of the specification as filed, the claims of the present divisional application, and a supplemental declaration, together with a letter explaining to each of the inventors why we asked them to re-sign the declaration. A copy of these letters (dated July 13, 2001) is enclosed (*Annex 4*), as well as the English translation thereof (*Annex 5*).
11. The text of these letters was identical for all of the inventors and all of the packages were sent registered mail requesting the parties to sign for the contents thereof.
12. In response to this letter, Mr. TOVEY replied on July 24, 2001, and sent to us the declaration, dated and signed (*Annex 6*).
13. Our letter to Mr. PLAVEC was returned to us on July 19, 2001, with the indication
"N'HABITE PAS À L'ADRESSE INDIQUÉE
RETOUR A L'ENVOYEUR",
which means :
"DOES NOT LIVE AT THE INDICATED ADDRESS

RETURN TO SENDER"

14. Having received Mr. PLAVEC's telephone number, on July 23rd, 2001, I left a message on the voice mail of Mr. PLAVEC, asking him to contact me. I left my telephone number, as well as my facsimile number at Ernest Gutmann – Yves Plasseraud S.A. on his voice mail. I also explained why we needed to contact him and the problem with the Declaration. I requested that he forward to us his new address in the U.S. He sent me his address on August 6th via facsimile, to which I sent the letter of July 13, 2001 with enclosures, on August 10. A copy of our letter to Mr. PLAVEC is enclosed (*Annex 7*). In response to this letter, Mr. PLAVEC signed and dated the Declaration on August 27, 2001, and then returned it to us (*Annex 8*).
15. Our registered mail of July 16, 2001 to Mr. MEYER was collected on July 18, 2001, as evidenced by the enclosed acknowledgement of receipt n° 6156 2081 5FR (*Annex 9*), which was translated from French into English.

The stamp on the Addressee's signature indicates that the package was collected by Mr. Meyer on July 18, 2001.

We also enclose a photocopy of the acknowledgement of receipt, which we have translated from French into English (*Annex 10*).
16. Since we did not receive any answer from Mr. MEYER, we resent to him the same package as indicated, with the same enclosures, on September 4, 2001. The mail was collected on September 6, 2001, as evidenced by the enclosed acknowledgement of receipt n° 6156 2268 8FR (*Annex 11*), which carries the same information as described above and in Annex 10, wherein the date when the mail was collected is indicated after the item "*Distribué le :*" (collected on :).
17. We then resent the same package to Mr. MEYER with the same enclosures on October 5, 2001. The mail was collected on October 9, 2001, as evidenced by the enclosed acknowledgement of receipt n° 6156 2426 4FR (*Annex 12*), which carries the same information as described above and in Annex 10, wherein the date when the mail was collected is indicated after the item "*Distribué le :*" (collected on :).
18. Mr. MEYER signed and dated the Declaration on November 5, 2001, and returned it to us (*Annex 13*).
19. Our registered mail of July 16, 2001 to Mr. BENOIT was not collected by the latter and was returned to us (*Annex 14*). On the back of the envelope, the acknowledgement of receipt indicates that the mail was not asked for. Indeed, the stamp

"NON RÉCLAMÉ
RETOUR À L'ENVOYEUR", means :

"NOT ASKED FOR
RETURN TO SENDER"

The other writings on this envelope have the following meaning :

"REÇU LE" (red stamp) means "RECEIVED ON", and indicates the date when we receive the envelope after the post returned it to us,

"*LETTRE*" means "LETTER",

"*RECOMMANDÉ AR*" means "REGISTERED WITH ACKNOWLEDGEMENT OF RECEIPT", and

"*Bureau de .*" (purple sticker) means "Office ." (this sticker has been scored out and we do not know the meaning of what is hand-written above it).

We also enclose as *Annex 15* a copy of the back of the envelope, on which we translated all the indications thereon.

20. We resent the same package as indicated in paragraph 19 to Mr. BENOIT, with the same letter and the same enclosures, on September 4, 2001, The mail was collected on September 6, 2001, as evidenced by the enclosed acknowledgement of receipt n° 6156 2267 4FR (*Annex 16*), which carries the same information as described above and in Annex 10, wherein the date when the mail was collected is indicated after the item "*Distribué le .*" (collected on :).
21. Therefore, we resent the same package to Mr. BENOIT for the third time on October 5, 2001. The mail was collected on October 13, 2001, as evidenced by the enclosed acknowledgement of receipt n° 6156 2427 4FR (*Annex 17*), which carries the same information as described above and in Annex 10, wherein the date when the mail was collected is indicated after the item "*Distribué le .*" (collected on :).
22. At the present date, we have not received the Declaration signed by Mr. BENOIT.
23. Concerning the last inventor, our registered mail of July 16, 2001 to Ms. MAGUIRE was not collected by the latter and was returned to us on August 6, 2001. This information is enclosed (*Annex 18*). On the back of the envelope, the acknowledgement of receipt indicates that the mail was not asked for, as attested by the same stamp already mentioned above, namely:

" <i>NON RÉCLAMÉ</i> <i>RETOUR À L'ENVOYEUR</i> ", which means :	"NOT ASKED FOR RETURN TO SENDER"
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The other writings on the envelope have the same meaning as described in the above paragraphs and in Annex 14.

We also enclose as *Annex 19* a copy of the back of the envelope, on which we translated all the indications thereon.
24. On September 4, 2001, we resent the same package to Ms. MAGUIRE, with the same letter and the same enclosures. This package was returned to us on September 25, 2001, for the same reason as the first one, i.e., it was not collected. This package is also enclosed (*Annex 20*), with the same indications as described above, and another hand-written phrase, namely: "*porte codée*", which means "door with entry code".
25. Finally, we resent the same package for the third time on October 5, 2001. This package was returned to us on October 29, 2001, as being not collected by the

addressee. This package is enclosed (Annex 21), with on the acknowledgement of receipt the indications:

" *NON RÉCLAMÉ*
RETOUR À L'ENVOYEUR ", which means :

"NOT ASKED FOR
RETURN TO SENDER"

26. As a summary of the above, it appears that despite our efforts, two inventors have not re-signed the Declaration:

- Mr. BENOIT, who has received our mail at least twice (on September 08 and October 13, 2001), and
- Ms. MAGUIRE, whom we could not reach.

These inventors have not signed the Declaration although we sent it three times at their last known address. Their last known address was set forth in the letter of July 12 (Annex 3) and, to the least of our knowledge, we have no other means to reach them.

27. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 22/11/01


Julia ANDRAL-ZIURYS

LIST OF ANNEXES

- Annex 1.** Letter sent to the client (LEB-TECH attention: Mrs. Lagneau) and one of the inventors, Mr. Tovey, with a copy of a New Declaration and Power of Attorney, by Anne DESAIX and Véronique MARCADÉ.
- Annex 2.** Facsimile to Ms. Lagneau, to remind her of our letter of May 31, 2001, and to suggest that our firm send the declarations to the inventors.
- Annex 3.** Copy of letters dated July 11 and 12 from Ms. VOULGARIS, working at CFPI Nufarm, in which she indicated that she knew the address of three of the inventors (those of Mr. TOVEY, Mr. MEYER, and Mr. BENOIT), but that neither she nor Mr. TOVEY knew the current address of Mr. PLAVEC and Ms. MAGUIRE, with their translation into English. The letter of July 12, 2001 also contained the telephone number of Mr. PLAVEC, now working in the US.
- Annex 4.** Copy of the registered letter (dated July 13, 2001) that was sent on around July 16, 2001 to the last known address of each of the inventors. The package included copy of the Declaration of 11/11/94, signed by the inventors, a copy of the specification as filed, the claims of the present divisional application, and a supplemental declaration, together with a letter explaining to each of the inventors why we asked them to re-sign the declaration.
- Annex 5.** Translation of the letter dated July 13, 2001, that was sent to the inventors.
- Annex 6.** Declaration dated and signed by Mr. TOVEY on July 23, 2001.
- Annex 7.** Copy of our letter of August 10, 2001, to Mr. PLAVEC, at his new address in the U.S.
- Annex 8.** Declaration signed and dated by Mr. PLAVEC on August 27, 2001.
- Annex 9.** Acknowledgement of receipt n° 6156 2081 5FR of our registered mail of July 16, 2001 to Mr. MEYER and collected on July 18, 2001.
- Annex 10.** Translation from French into English of Annex 9.
- Annex 11.** Acknowledgement of receipt n° 6156 2268 8FR of the 2nd sending of September 4, 2001, of the same package to Mr. MEYER with the same enclosures, and collected on September 6, 2001.
- Annex 12.** Acknowledgement of receipt n° 6156 2426 4FR of the 3rd sending of October 5, 2001, of the same package to Mr. MEYER with the same enclosures, collected on October 9, 2001.

- Annex 13.** Declaration dated and signed by Mr. MEYER on November 5, 2001.
- Annex 14.** Our registered mail of July 16, 2001 to Mr. BENOIT that was not collected by the latter and returned to us. On the back of the envelope, the acknowledgement of receipt indicates that the mail was not asked for.
- Annex 15.** Translation from French into English of the back of the envelope mentioned in Annex 14.
- Annex 16.** Acknowledgement of receipt n° 6156 2267 4FR of our registered mail of September 4, 2001, to Mr. BENOIT, and collected on September 8, 2001.
- Annex 17.** Acknowledgement of receipt n° 6156 2427 4FR of our registered mail of October 5, 2001, to Mr. BENOIT, and collected on October 13, 2001.
- Annex 18.** Our registered mail of July 16, 2001 to Ms. MAGUIRE, which was not collected by the latter and was returned to us on August 6, 2001. On the back of the envelope, the acknowledgement of receipt indicates that the mail was not asked for.
- Annex 19.** Translation from French into English of the back of the envelope mentioned in Annex 18.
- Annex 20.** 2nd sending of the same package to Ms. MAGUIRE on September 4, 2001, with the same letter and the same enclosures. This package was returned to us on September 25, 2001, for the same reason as the first one, i.e., it was not collected.
- Annex 21.** 3rd sending of the same package to Ms. MAGUIRE on October 5, 2001. This package was returned to us on October 29, 2001, as being yet again not collected by the addressee.



LIST OF ANNEXES

RECEIVED
DEC 12 2001
TECH CENTER 1600/2900 6

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- Annex 5.** Translation of the letter dated July 13, 2001, that was sent to the inventors.
- Annex 6.** Declaration dated and signed by Mr. TOVEY on July 23, 2001.
- Annex 7.** Copy of our letter of August 10, 2001, to Mr. PLAVEC, at his new address in the U.S.
- Annex 8.** Declaration signed and dated by Mr. PLAVEC on August 27, 2001.
- Annex 9.** Acknowledgement of receipt n° 6156 2081 5FR of our registered mail of July 16, 2001 to Mr. MEYER and collected on July 18, 2001.
- Annex 10.** Translation from French into English of Annex 9.
- Annex 11.** Acknowledgement of receipt n° 6156 2268 8FR of the 2nd sending of September 4, 2001, of the same package to Mr. MEYER with the same enclosures, and collected on September 6, 2001.
- Annex 12.** Acknowledgement of receipt n° 6156 2426 4FR of the 3rd sending of October 5, 2001, of the same package to Mr. MEYER with the same enclosures, collected on October 9, 2001.

- Annex 13.** Declaration dated and signed by Mr. MEYER on November 5, 2001.
- Annex 14.** Our registered mail of July 16, 2001 to Mr. BENOIT that was not collected by the latter and returned to us. On the back of the envelope, the acknowledgement of receipt indicates that the mail was not asked for.
- Annex 15.** Translation from French into English of the back of the envelope mentioned in Annex 14.
- Annex 16.** Acknowledgement of receipt n° 6156 2267 4FR of our registered mail of September 4, 2001, to Mr. BENOIT, and collected on September 8, 2001.
- Annex 17.** Acknowledgement of receipt n° 6156 2427 4FR of our registered mail of October 5, 2001, to Mr. BENOIT, and collected on October 13, 2001.
- Annex 18.** Our registered mail of July 16, 2001 to Ms. MAGUIRE, which was not collected by the latter and was returned to us on August 6, 2001. On the back of the envelope, the acknowledgement of receipt indicates that the mail was not asked for.
- Annex 19.** Translation from French into English of the back of the envelope mentioned in Annex 18.
- Annex 20.** 2nd sending of the same package to Ms. MAGUIRE on September 4, 2001, with the same letter and the same enclosures. This package was returned to us on September 25, 2001, for the same reason as the first one, i.e., it was not collected.
- Annex 21.** 3rd sending of the same package to Ms. MAGUIRE on October 5, 2001. This package was returned to us on October 29, 2001, as being yet again not collected by the addressee.

BDS
17283/675

ANNEX 1

Letter sent to the client (LEB-TECH attention: Mrs. Lagneau) and one of the inventors, Mr. Tovey, with a copy of a New Declaration and Power of Attorney, by Anne DESAIX and Véronique MARCADÉ.



ERNEST GUTMANN - YVES PLASSERAUD S.A.
SOCIÉTÉ DE CONSEILS EN PROPRIÉTÉ INDUSTRIELLE

PARIS (SIÈGE) :
3, RUE CHAUVEAU-LAGARDE
F - 75008 PARIS
TÉL : 33 (0)1 44 51 18 00
FAX : 33 (0)1 42 66 08 90
e-mail : info@egyp.fr

LYON (AGENCE) :
62, RUE DE BONNEL
F - 69448 LYON CEDEX 03
TÉL : 33 (0)4 72 84 97 60
FAX : 33 (0)4 72 84 97 65

ALICANTE (AGENCE) :
PLAZA DEL AYUNTAMIENTO 2-2º-2º
ES - 03002 ALICANTE
TÉL : 34 965 230 611
FAX : 34 965 230 639

VIA FACSIMILE
+ CONFIRMATION

URGENT

LEB-TECH

28, Boulevard Camélinat

92333 GENNEVILLIERS

Attention : Mrs. LAGNEAU

VOIRE REFERENCE :

NOTRE REFERENCE :

LE

B1608ACA – AD/VMA/MNH

May 31, 2001

Re: "MONOCLONAL ANTIBODY ALPHA IFN"
US Patent Application N° 09/240,675
filed on 02/02/1999
Divisional Application of US Serial N° 08/307,588
Applicant: LABORATOIRE EUROPEEN DE BIOTECHNOLOGIE S.A.

BREVETS

Ernest GUTMANN, cpl *
Anne DESAIX, cpl *
Carol ALMOND-MARTIN *⁽¹⁾
Julia ANDRAL-ZIURYS *
Florence LAZARD, cpl
Jeanne VAILLANT, cpl *
Lionnel BIROTHEAU
Véronique MARCADÉ
Denis BOURGAREL
Carole SELLIN⁽¹⁾

MARQUES, DESSINS

ET MODÈLES

Yves PLASSERAUD, cpl *
Martine DEHAUT, cpl *
Virginie ZANCAN, cpl *
Nathalie PACAUD
Benjamin FONTAINE⁽²⁾
Christophe PELÈSE

DOCUMENTATION

ET VIEILLE TECHNOLOGIQUE

Jean-Charles THEODET

Dear Mrs. Lagneau,

We have received from our American associate a new Official Action issued on January 31, 2001, by the United States Patent and Trademark Office, in connection with the above-referenced application, and of which you will find attached a copy.

We are drafting a response to this Official action and shall shortly submit it to you.

Meanwhile, we are sending you beforehand a new "Declaration and Power of Attorney" that is requested in paragraph 11 of the present Action. Could you please have it signed by all the inventors, if necessary on separate copies, and send it back to us as soon as possible? In the eventual case where some of the inventors cannot be located or refuse to participate, the application may be made by the other inventors on behalf of themselves and the omitted inventors. In such a case, the following items are required:

- 1. A declaration signed by every available inventor, with the signature block of the nonsigning inventor left blank.**
- 2. Proof that the nonsigning inventor cannot be found or reached after diligent effort.**
- 3. The last known address of the nonsigning joint inventor.**

*mandataire agréé OEB/EPO

co: ⁽²⁾US patent attorney
européen en marques
OHM/OHIM

⁽¹⁾Agence de Lyon
⁽²⁾Agence d'Alicante

SOCIÉTÉ ANONYME
AU CAPITAL DE 3 000 000 F
RCS PARIS B 332 417 500
APE 741 A

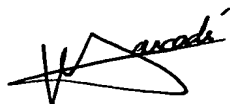
Acquisition et défense des droits, stratégie de protection, liberté d'exploitation
et recherches de disponibilité, oppositions, consultations, contrats et audits

In this latter case, please let us know as soon as possible, so that we will provide you with an appropriate affidavit for items 2 and 3.

The deadline for responding to this outstanding action is set to expire now on **June 30, 2001**, but is still extendable for one month upon payment of government fees.

Of course, should you have any questions, please do not hesitate to contact us.

Yours sincerely,



Véronique MARCADÉ


Anne DESAIX

Encl. : Declaration and Power of Attorney

Copy : Mr. Tovey (LEB-TECH)

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR,
WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON**

(Attorney Docket No. 017283/0123)

the specification of which (check one)

☐ Is attached hereto.

☒ Was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
92400902.0	European	31/March/1992	Yes	NO

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

STEPHEN A. BENT	Reg. No. 29,768
DAVID A. BLUMENTHAL	Reg. No. 26,257
BETH A. BURROUS	Reg. No. 35,087
ALAN I. CANTOR	Reg. No. 28,163
WILLIAM T. ELLIS	Reg. No. 26,874
JOHN J. FELDHAUS	Reg. No. 28,822
MICHAEL D. KAMINSKI	Reg. No. 32,904
LYLE K. KIMMS	Reg. N . 34,079
KENNETH E. KROSIN	Reg. No. 25,735

JOHNNY A. KUMAR	Reg. No. 34,649
JACK LAHR	Reg. No. 19,621
GLENN LAW	Reg. No. 34,371
PETER G. MACK	Reg. No. 26,001
STEPHEN B. MAEBIUS	Reg. No. 35,264
BRIAN J. MC NAMARA	Reg. No. 32,789
SYBIL MELOY	Reg. No. 22,749
RICHARD C. PEET	Reg. No. 35,792
GEORGE E. QUILLIN	Reg. No. 32,792
ANDREW E. RAWLINS	Reg. No. 34,702
BERNHARD D. SAXE	Reg. No. 28,665
CHARLES F. SCHILL	Reg. No. 27,590
RICHARD L. SCHWAAB	Reg. No. 25,479
MICHELE M. SIMKIN	Reg. No. 34,717
HAROLD C. WEGNER	Reg. No. 25,258

to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

I request that all correspondence be directed to:

Bernhard D. Saxe
Foley & Lardner
At Washington Harbour
3000 K Street, NW, Suite 500
Washington, DC 2000

Telephone: 202-672-5472
Facsimile: 202-672-5399

I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Name of first inventor	<u>Patrick BENOIT</u>
Residence	<u>Paris, France</u>
Citizenship	<u>FRANCE</u>
Post Office Address	<u>24, rue Jonquoy, F-75014 Paris, France</u>
Inventor's signature	<u></u>
Date	<u></u>

Name of second inventor	<i>Francois MEYER</i>
Residence	<i>Paris, France</i>
Citizenship	<i>LUXEMBOURG</i>
Post Office Address	<i>14, square Adanson, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of third inventor	<i>Deborah MAGUIRE</i>
Residence	<i>Paris, France</i>
Citizenship	<i>AUSTRALIA</i>
Post Office Address	<i>24, rue Maitre-Albert, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of fourth inventor	<i>Ivan PLAVEC</i>
Residence	<i>Fresnes, France</i>
Citizenship	<i>CROATIA</i>
Post Office Address	<i>1, allée du Capitaine-Dupont F-92260 Fresnes, France</i>
Inventor's signature	
Date	
Name of fifth inventor	<i>Michael G. TOVEY</i>
Residence	<i>Paris, France</i>
Citizenship	<i>GREAT BRITAIN</i>
Post Office Address	<i>6, rue des Quatrefages, F-75005 Paris, France</i>
Inventor's signature	
Date	

un
17283/615

ANNEX 2

Facsimile to Ms. Lagneau, to remind her of our letter of May 31, 2001, and to suggest that our firm send the declarations to the inventors.

FACSIMILE TRANSMISSION

DE/FROM: Anne DESAIX/Véronique MARCADÉ	DATE : June 21, 2001
A/TO : LEB-TECH <u>Attention : Mrs LAGNEAU</u> c/o CFPI Fax : 01 40 85 54 59	
VOS/YOUR REF.	NOS/OUR REF. B1608ACA-AD/VMA
Nombre de pages, y compris la page de garde : Number of pages, including this cover page 3	

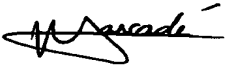
Re: "MONOCLONAL ANTIBODY ALPHA IFN"
US Patent Application N° 09/240,675
filed on 02/02/1999
Divisional Application of US Serial N° 08/307,588
Applicant: LABORATOIRE EUROPEEN DE BIOTECHNOLOGIE S.A.

Dear Mrs. Lagneau,

We sent you together with our letter of May 31, 2001 (copy of which is attached), a new "Declaration and Power of Attorney" that has to be signed by all the inventors. As the next deadline for filing a response to the outstanding Action is June 30, 2001, we would appreciate receiving the referenced declaration as soon as possible, in order to allow us to timely file the response without an additional extension of time.

We suggest, should you wish so, that we could send the declaration to each one of the inventors, by registered mail. Please let us have your instructions as soon as possible.

Best regards.


Véronique MARCADÉ


Anne DESAIX

Attachment : copy of letter dated May 31, 2001

En cas de mauvaise transmission, veuillez nous en informer immédiatement
In case of bad printing or missing page, please inform us immediately.

EMISSION

ÉMETTEUR
RÉCEPTEUR
DOCUMENT
COMMUNICATION

Número: 0142660890
Nom: GUTMANN-PLASSERAUD
Número: 0140855459
Nom: 33 1 40855459
172_1351.FAX
Page(s): 3
Durée: 1'39 à 9600 en mode Normal
Résultat: Correct

21/06/01 13:51:16

Fax émis par: 0142660890

GUTMANN-PLASSERAUD

le 21/06/01 13:49 R4 NORM Pg: 1/3

ERNEST GUTMANN-YVES PLASSERAUD S. A.
3 rue Chauveau-Lagarde - 75008 PARIS (FRANCE)

☎ 33 (1) 44 51 18 00
Fax 33 (1) 42 66 08 90

FACSIMILE TRANSMISSION

DE/FROM: Anne DESAIX/Véronique MARCADÉ		DATE: June 21, 2001
A/TO: LEB-TECH <u>Attention : Mrs LAGNEAU</u> c/o CFPI Fax : 01 40 85 54 59		
VOS/YOUR REF.		NOS/OUR REF. B1608ACA-AD/VMA
Nombre de pages, y compris la page de garde : Number of pages, including this cover page : 3		

Re: "MONOCLONAL ANTIBODY ALPHA IFN"
US Patent Application N° 09/240,675
filed on 02/02/1999
Divisional Application of US Serial N° 08/307,588
Applicant: LABORATOIRE EUROPEEN DE BIOTECHNOLOGIE S.A.

Dear Mrs. Lagneau,

We sent you together with our letter of May 31, 2001 (copy of which is attached), a new "Declaration and Power of Attorney" that has to be signed by all the inventors. As the next deadline for filing a response to the outstanding Action is June 30, 2001, we would appreciate receiving the referenced declaration as soon as possible, in order to allow us to timely file the response without an additional extension of time.

We suggest, should you wish so, that we could send the declaration to each one of the inventors, by registered mail. Please let us have your instructions as soon as possible.

Best regards.


Véronique MARCADÉ


Anne DESAIX

Attachment : copy of letter dated May 31, 2001

En cas de mauvaise transmission, veuillez nous en informer immédiatement
In case of bad printing or missing page, please inform us immediately.

Les informations contenues dans cette télécopie sont strictement confidentielles et ne sont réservées qu'au destinataire. L'emploi de ce document (ou de son contenu) par toute autre personne est strictement interdit. Si vous recevez cette télécopie par erreur, merci de bien vouloir nous le faire savoir par retour de télécopie ou par téléphone.
This transmission contains confidential information intended only for the use of the addressee. The use of this document (or its contents) by anyone else is strictly prohibited. If you have received this document in error, we thank you to notify us immediately by telephone or return fax.

BDS
17283/123

ANNEX 3

Copy of letters dated July 11 and 12 from Ms. VOULGARIS, working at CFPI Nufarm



CFPI Nufarm

Gennevilliers, le 12 Juillet 2001

E G Y P

3, rue Chauveau Lagarde,
75008 PARIS

V/Réf. : **V/Réf : B1608ACA - AD/VMA/MNH**
N/Réf. : **ML/MV/443/2001**

A l'attention de Mme V. MARCADE

Objet : MONOCLONAL ANTIBODY ALPHA FIN
Us Patent Application N°09/240, 675 filed on 02/02/1999
Divisional Application of US Serial n°08/307, 588
Applicant : Laboratoire Européen de Biotechnologie

Madame,

Je vous prie de bien vouloir trouver ci-joint les dernières informations concernant les coordonnées des inventeurs du brevet référencé en objet pour l'envoi de formalités :

Nous avons pu identifier la nouvelle adresse de Mr François MEYER :

- **François MEYER**
3, Place du Panthéon - 75005 PARIS
(t : dom. : 01 55 71 85 11)
- **Patrick BENOIT**
24, rue Jonquoy - 75014 PARIS
(t : dom. : 01 45 42 09 30)
- **Michael TOVEY**
7, rue Lagrange - 75005 PARIS
(t : bur : 01 49 58 34 34)
- Pour ce qui concerne Mr Ivan PLAVEC, nous avons pu obtenir les informations qui nous indiquent qu'il travaille actuellement aux Etats Unis (CALIFORNIE) et dont nous avons uniquement les coordonnées téléphoniques mais que nous n'avons pas pu joindre :
(t : +1 650 552 0729)

Nous n'avons aucune information quant à l'adresse personnelle ou le lieu de travail de Mme Deborah MAGUIRE à l'exception de l'adresse suivante : 24, rue Maître Albert - 75005 Paris à laquelle elle n'habite plus.

Souhaitant que ces recherches répondent à vos attentes, nous restons à votre entière disposition pour tout renseignement complémentaire.

Veuillez agréer, Madame, l'expression de mes meilleures salutations.

P/o Voulgaris,

Martin LAGNEAU

28, boulevard Camélinat - BP 75 - 92233 GENNEVILLIERS Cedex (France)
Tél. : 01 40 85 50 50 - Télécopieur : 01 47 92 25 45 - Télex 610.626 CFPI GNVL
Société Anonyme au capital de 35 404 375 francs
R.C.S. Nanterre 552 029 069 - SIRET 552 029 069 00020 • N° TVA CEE : FR. 08552029068

GAILLON 27600
Noire-Dame-dn-la-Garenne
Tél. 02 32 64 74 00
Télécopieur 02 32 53 83 62

Usines
MULHOUSE 68200
40, rue Eugène Ducretet
Tél. 03 89 33 16 00
Télécopieur 03 89 33 16 81

DE:CFPI JURIDIQUE 12-JUL-01 14:42

33 1 40855459

A:33 0 1 42 66 08 90

PAGE:01

Le 12/07/01 14:53 A4 NORM Pg:1/2

fax reçu de: 33 1 40855459

Annex 3 :

Translation of the letter from CFPI Nufarm, dated July 12, 2001

Re: **MONOCLONAL ANTIBODY ALPHA FIN**
US Patent Application N° 09/240,675 filed on February 2, 1999
Divisional Application of US Serial No. 08/307,588
Applicant: Laboratoire Européen de Biotechnologie

Madam,

You will find enclosed the latest information concerning the addresses of the inventors of the above-referenced patent to complete the formalities:

We could find the new address of Mr. François MEYER:

- François MEYER
3, Place du Panthéon – 75005 PARIS
(☎ home : 01 55 71 85 11)
- Patrick BENOIT
24, rue Jonquoy – 75014 PARIS
(☎ home : 01 45 42 09 30)
- Michael TOVEY
7, rue Lagrange – 75005 PARIS
(☎ office : 01 49 58 34 34)

Concerning Mr. Ivan PLAVEC, we could only obtain information that indicates that he currently works in the United States (California). We only have his phone number but we could not reach him:
(☎: +1 650 552 0729).

We have no information about the personal address nor the place of work of Ms. Deborah MAGUIRE except the following address: 24, rue Maître Albert – 75005 Paris, where she does not live anymore.

We hope that this information will respond to your inquiries and we rest entirely at your disposal for any further information.

Sincerely yours.

Martine Lagneau



CFPI Nufarm

Gennevilliers, le 11 Juillet 2001

E G Y P

**3, rue Chauveau Lagarde,
75008 PARIS**

V/Réf. :

N/Réf. : **ML/MV/442/2001**

V/Réf : B1608ACA - AD/VMA/MNH

A l'attention de Mme V. MARCADE

Objet :

MONOCLONAL ANTIBODY ALPHA FIN

Us Patent Application N°09/240, 675 filed on 02/02/1999

Divisional Application of US Serial n°08/307, 588

Applicant : Laboratoire Européen de Biotechnologie

Madame,

Pour faire suite à vos courriers du 31/05/2001, et relances fin juin 2001, à nos différents entretiens téléphoniques notamment du 5 et 10 juillet 2001, nous vous confirmons que nous n'avons pas pu obtenir de plus amples informations concernant les coordonnées de 2 de 5 inventeurs du brevet référencé en objet.

En effet, nous n'avons pas pu localiser les inventeurs suivants :

Ms Deborah MAGUIRE

Mr Ivan PLAVEC.

Nous restons à votre entière disposition pour tout renseignement complémentaire,

Dans l'attente de vous lire,

Veuillez agréer, Madame, l'expression de mes meilleures salutations.

M. Bulgare

Martine LAGNEAU

Certificat N° 0935-ED1C



**28, boulevard Camélinat - BP 75 - 92233 GENNEVILLIERS Cedex (France)
Tél. : 01 40 85 50 50 - Télécopieur : 01 47 92 25 45 - Télex 610.626 CFPI GNVL**

**Société Anonyme au capital de 35.404 375 francs
R.C.S. Nanterre 552 028 068 - SIRET 552 028 068 00020 - N° TVA CEE : FR. 08552028068**

GAILLON 27600

**Noire-Dame-de-la-Garenne
Tél. 02 32 64 74 00
Télécopieur 02 37 53 83 62**

Usines

MULHOUSE 68200

**49, rue Eugène Ducretet
Tél. 03 89 33 16 60
Télécopieur 03 89 33 16 61**

PAGE:01

A:001426608990

33 1 40855459

DE:CFPI JURIDIQUE

11-JUL-01 17:33

Le 11/07/01 17:44 A4 NORM Pg:1/1

Fax reçu de: 33 1 40855459

Annex 3 :

Translation of the letter from CFPI Nufarm, dated July 11, 2001

Re: **MONOCLONAL ANTIBODY ALPHA FIN**
US Patent Application N° 09/240,675 filed on February 2, 1999
Divisional Application of US Serial No. 08/307,588
Applicant: Laboratoire Européen de Biotechnologie

Madam,

Following your letter of 31/05/2001 and reminders at the end of June 2001, our different telephone conversations notably of July 5 and 10, 2001, we confirm that we could not obtain any further information concerning the address of 2 of 5 inventors of the above-referenced patent.

Indeed, we could not locate the following inventors:

- Ms. Deborah MAGUIRE
- Mr. Ivan PLAVEC.

We stay entirely at your disposal should you require any further information.

Looking forward to reading from you,

Yours sincerely

Martine Lagneau

VERIFICATION OF TRANSLATION

I, Julia ANDRAL-ZIURYS, working at ERNEST GUTMANN-YVES PLASSERAUD S.A., 3 rue Chauveau-Lagarde, 75008 Paris (France),

declare that I am conversant with the French and English languages and that to the best of my knowledge and belief the following is a true translation of Annex 3 : letters dated July 11 and 12 from Ms. VOULGARIS, working at CFPI Nufarm.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application or any patent issuing thereon.

Paris, November 22, 2001

Signature:

A handwritten signature in cursive script, appearing to read "Julia Andral-Ziurys".

Julia ANDRAL-ZIURYS

22/11/01

17283/125

ANNEX 4

Copy of the registered letter (dated July 13, 2001) that was sent on around July 16, 2001 to the last known address of each of the inventors. The package included copy of the Declaration of 11/11/94, signed by the inventors, a copy of the specification as filed, the claims of the present divisional application, and a supplemental declaration, together with a letter explaining to each of the inventors why we asked them to re-sign the declaration.



ERNEST GUTMANN - YVES PLASSERAUD S.A.
SOCIÉTÉ DE CONSEILS EN PROPRIÉTÉ INDUSTRIELLE

PARIS (SIÈGE) :
3, RUE CHAUVÉAU-LAGARDE
F - 75008 PARIS
TÉL. : 33 (0)1 44 51 18 00
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ES - 03002 ALICANTE
TÉL. : 34 965 230 611
FAX : 34 965 230 639

Par RECOMMANDÉ A/R

Monsieur Ivan PLAVEC
1 allée du Capitaine-Dupont

92260 FRESNES

VOTRE RÉFÉRENCE :

NOTRE RÉFÉRENCE :

LE

B1608ACA – JAZ/VMA/PAD

13 juillet 2001

Objet : Brevet américain n° US 5,919,453 du 6 juillet 1999
Demande divisionnaire n° 09/240,675 déposée le 2 février 1999
au nom de MEDISUP INTERNATIONAL N.V.
Inventeurs : BENOIT, MEYER, MAGUIRE, PLAVEC, TOVEY

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Julia ANDRAL-ZIURYS °
Florence LAZARD, cpi
Jeanne VAILLANT, cpi * °
Véronique MARCADÉ
Denis BOURGAREL
Carole SELLIN (1)

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ET MODÈLES

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Benjamin FONTAINE (1)
Christophe PELÈSE

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ET VEILLE TECHNOLOGIQUE

Jean-Charles THEODET

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° US patent attorney

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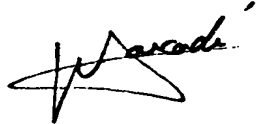
° Agence d'Alicante

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RCS PARIS B 332 417 500
APE 741 A

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Véronique MARCADÉ



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ERNEST GUTMANN - YVES PLASSERAUD S.A.
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Par RECOMMANDE A/R

Monsieur Michael G. TOVEY
7 rue Lagrange

75005 PARIS

VOIRE REFERENCE :

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ET MODÈLES

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DOCUMENTATION

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Jean-Charles THEODET

*mandataire agréé OEB/EPO

*US patent attorney

*convention de coopération en marques
OHIM/OHIM

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Par RECOMMANDÉ A/R

Monsieur François MEYER
3 Place du Panthéon

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Monsieur Patrick BENOIT
24 rue Jonquoy

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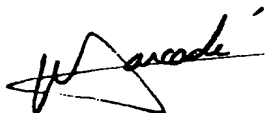
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Par RECOMMANDÉ A/R

Madame Deborah MAGUIRE
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75005 PARIS

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DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE 1 INTERFERON

the specification of which (check one)

☐ is attached hereto

☒ was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
92400902.0	European	31/March/1992	Yes

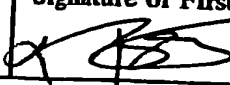
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; John J. Feldhaus, Reg. No. 28,822; Donald D. Jeffery, Reg. No. 19,980; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Neil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 22,115; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115.

Send all correspondence to **FOLEY & LARDNER**, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696.
Address telephone communications to Bernhard D. Saxe at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor Patrick BENOIT		Signature of First or Sole Inventor 	Date 11/11/94
Residence Address Paris, France		Country of Citizenship FRANCE	
Post Office Address 24, rue Jonquoy, F-75014 Paris, France			

Signatures should conform to names as typewritten. ☒ Additional inventors on attached Page 2.

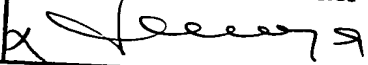
Full Name of Second Inventor Francois MEYER	Signature of Second Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 14. square Adanson, F-75005 Paris, France		


Deborah


Full Name of Third Inventor Deborah-MAGUIRE	Signature of Third Inventor <i>[Signature]</i>	Date 11/14/94
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 24, rue Maitre-Albert, F-75005 Paris, France		

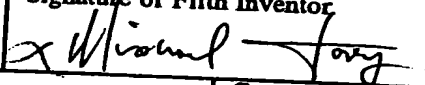
Full Name of Fourth Inventor Ivan PLAVEC	Signature of Fourth Inventor <i>[Signature]</i>	Date 11/14/94
Residence Address Fresnes, France	Country of Citizenship FRANCE CROATIA	
Post Office Address 1, allée du Capitaine-Dupont F-92260 Fresnes, France		

Full Name of Fifth Inventor Michael G. TOVEY	Signature of Fifth Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
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Post Office Address 6, rue des Quatrefages, F-75005 Paris, France		

Full Name of Second Inventor Francois MEYER	Signature of Second Inventor 	Date 11.XI.94
Residence Address Paris, France	Country of Citizenship FRANCE LUXEMBOURG	
Post Office Address 14, square Adanson, F-75005 Paris, France		

Full Name of Third Inventor Deborah MAGUIRE	Signature of Third Inventor 	Date
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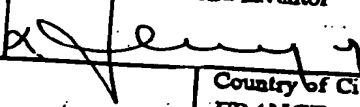
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
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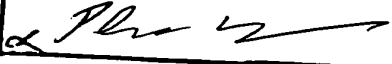
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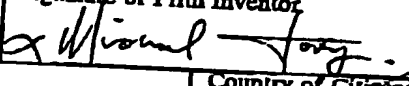
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Docket No. 17283/117/GUPL

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*Revencliations de la demande
discriminaire*

IN THE CLAIMS:

Please delete claims 1-22 and insert the following new claims:

--23. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 27 to amino acid residue 427 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12 (deposited at the ECACC under no. 92022605).

24. A peptide or polypeptide as claimed in claim 23, consisting of amino acid residue 27 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof.

25. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 1 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12.

26. An analogue of a peptide or polypeptide as claimed in claim 23, which is derived from said peptide or polypeptide by substitution of one or more amino acid residues and which retains the ability to specifically bind to monoclonal antibody 64G12.

27. A method of producing a monoclonal antibody, comprising immunizing an animal with a peptide or polypeptide as claimed in claim 23, fusing spleen cell from the immunized animal with myeloma cells, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide.

28. A method of producing a monoclonal antibody, comprising contacting stimulated B-lymphocytes *in vitro* with a peptide or polypeptide according to claim 23, fusing the resultant B-lymphocytes with B-lymphocytes immortalized with Epstein-Barr

virus, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide. --

IN THE ABSTRACT


Please insert the Abstract provided on the attached sheet.

REMARKS

The Examiner is respectfully requested to enter the above amendments prior to examination of the instant application. Support for the amendments is present throughout the specification, in particular at pages 10-11.

Respectfully submitted,

February 2, 1999
Date


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ABSTRACT OF THE DISCLOSURE

A monoclonal antibody is provided which is directed against the human interferon type I receptor (IFN-R), which recognizes the extracellular domain of the human IFN-R and which has neutralizing capacity against the biological properties of human type I-IFN. Diagnostic and therapeutic applications for the monoclonal antibody also are provided.

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN : alpha (α), beta (β) and gamma. IFN α and IFN β together are known as the type I interferon.

Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33:251, 1986).

The human IFN α locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, α II or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFN α genes (Weissmann and Weber 1986).

The subtypes of IFN α have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78:2848, 1981) and have the same cellular receptor (Agnat M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFN β) is encoded by a single gene which has approximately 50% homology with the IFN α genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

The interferon gamma (IFN gamma) is also encoded by a single copy, which has little homology with the IFN α and IFN β genes. The receptor for IFN gamma is distinct from the receptor of the α and β interferons.

For the purpose of the present invention the receptor of α and β classes of IFN will be designated IFN-R. This represents natural type I receptor. The group of proteins forming natural interferon α will be designated IFN α , and type I-IFN will represent both natural IFN α , IFN ω , and IFN β .

Despite the fact that interferon is a potent antiviral agent, there is considerable evidence to suggest, that many of the characteristic symptoms of acute virus diseases such as upper respiratory tract infections are caused by an overproduction of interferon alpha. Furthermore, IFN alpha has been shown to contribute to the pathogenesis of certain chronic virus infections in experimental animals and the available evidence suggests that this is also the case for certain human chronic virus diseases such as those due to measles virus.

The interferons α are also potent immunoregulatory molecules which stimulate polyclonal B-cell activation, enhance NK cell cytotoxicity, inhibit T-cell functions, and modulate the expression of the major histocompatibility complex (MHC) class I antigens, all of which are implicated in the induction of autoimmunity and in graft rejection. The abnormal production of interferon α is associated with a number of autoimmune diseases and inflammatory disorders including systemic lupus erythematosus (SLE), type I diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, the acquired immunodeficiency syndrome (AIDS) and severe

combined immunodeficiency diseases . The presence of interferon α in the serum of patients with systemic lupus is correlated with both the clinical and humoral signs of increased disease activity. The production of interferon α in HIV positive subjects is also highly predictive of disease evolution.

Administration of interferon α has been reported to exacerbate underlying disease in patients with psoriasis and multiple sclerosis and to induce a SLE like syndrome in patients without a previous history of autoimmune disease. Interferon α has also been shown to induce glomerulonephritis in normal mice and to accelerate the outset of the spontaneous autoimmune disease of NZB/W mice.

Interferon α is also produced during the course of graft-versus-host disease (GVHD) in parallel with the enhanced NK cell activity characteristic of systemic GVHD. Interferon α is the principal modulator of NK cell cytotoxicity and administration of interferon α has been shown to enhance the intestinal consequences of GVHD in normal mice.

The object of the present invention is to provide new antagonists against the biological activities of the human type I-IFN. These antagonists could be used for therapeutical, including prophylaxis purposes, in cases where the type I-IFN (IFN α/β) is abnormally produced and when this abnormal production is associated with pathological symptoms. Such antagonists could also be used for the diagnosis of various diseases or for the study of the evolution of such diseases.

In order to define such antagonists, the inventors have taken into account the fact that the human natural type I-IFN is in fact constituted of a mixture of

interferons (subspecies) and the fact that the composition of this association of different subtypes of interferons varies both quantitatively and qualitatively.

Some natural interferons, such as the ones secreted by Namalwa cells (Namalwa interferon) or leukocyte (leukocyte interferon) have been studied in detail (N.B. Finter and K.H. Fautes, Interferon 2, 1980, p. 65-79 I. Gresser Editor Academic Press ; K. Cantell et al, Interferon 1, 1979 p. 2-25, I. Gresser Editor Academic Press) and were used by the inventors to define natural type I interferons.

In some pathological cases, like AIDS, interferons having some special properties have been described (O.T. Preble et al, Annals of New-York Academy of Sciences p. 65-75). This interferon involved in pathological cases like AIDS nevertheless binds to the same receptor, as described above.

One object of the present invention is to provide an antagonist of the type I-IFN, which would be able to inhibit or neutralize, to a determined extent, the biological properties of the human type I-IFN, that is to say, to neutralize in vivo a mixture of α , β , ω subspecies.

Accordingly the inventors have defined antibodies, especially monoclonal antibodies, which have the property of being antagonists to the type I-IFN. These antibodies are directed against the human type I-IFN receptor.

The invention thus also concerns the use of the monoclonal antibodies for the preparation of pharmaceutical compositions, useful for the treatment of symptoms associated with the abnormal production of

type I-IFN. These monoclonal antibodies are also appropriate for the preparation of diagnosis reagents.

A monoclonal antibody according to the present invention is directed against the human type I-interferon receptor (IFN-R) and is characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

The ability to neutralize the biological properties of type I-IFN can be estimated as a function of the capacity of the monoclonal antibody to neutralize the antiviral activity of the type I-IFN. Such a test is relevant in order to determine whether the antibody assayed is included within the scope of the invention, although it is clear that the biological properties of type I-IFN are not limited to its antiviral properties. Detailed procedures are given in the examples in order to enable to perform such a test of the antiviral activity. The cells tested can advantageously be Daudi-cells, which affinity for the type I-IFN is well known. The main steps of such a test would consist in :

- incubating a determined concentration of human cells responsive to human type I-IFN, with human type I-IFN in the presence of a determined concentration of monoclonal antibodies to be assayed, for a time sufficient to allow the formation of a complex between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the incubated cells with a determined virus, in a determined concentration,
- washing the cells,
- resuspending the cells in culture medium,
- incubating for a time sufficient to allow virus replication ;
- lysing the cells ;
- measuring the virus replication, or measuring the inhibition of the cytopathic effect.

The ability of the monoclonal antibodies of the invention to neutralize the biological properties of the human type I-IFN can be modulated as a function of the dose of antibodies used. Accordingly a 100% inhibition of the biological properties, or a partial inhibition can be obtained.

According to another embodiment of the present invention, the monoclonal antibodies directed against the human type I-IFN receptor, are further characterized by the fact that they are capable of inhibiting the binding of a human type I-IFN, to the human IFN-R.

A monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN to its receptor, can be selected by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies or a hybridoma culture supernatant containing monoclonal antibodies to be assayed, with human cells capable of harboring IFN-R ;
- adding labelled human type I-IFN, in a determined concentration, to the above preincubated medium ;

- incubating the medium containing the human cells, the monoclonal antibodies and the labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the labelled type I-IFN by counting the amount of attached labelled type I-IFN.

Some of the monoclonal antibodies of the invention, have also the capacity to neutralize the antiproliferative properties of the human type I-IFN. This property can also be assayed on Daudi cells, by performing the following steps :

- allowing cells to grow in presence of human type IFN and determined concentration of mAb ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the human type I-IFN.

One property of a monoclonal antibody according to the invention resides in its capacity to recognize the extracellular domain of the human IFN receptor. This property of the monoclonal antibody can be assayed on human cells bearing the natural human receptor but also on the extracellular domain of a recombinant IFN-R such as expressed in a procaryotic cell, for instance in E.coli or a recombinant IFN-R such as expressed in a eucaryotic cell such as mamalian cell for instance a CHO-cell.

This receptor can indeed present different properties, depending on the fact that it is produced in a procaryotic or eucaryotic cell and accordingly

depending on the fact that the post-translational maturation occurred or not. The inventors interestingly showed that relevant assays, to evaluate the capacity of a monoclonal antibody according to the invention i.e. to recognize the cellular IFN-R, can be performed on a recombinant receptor expressed in mamalian cells. As a matter of fact, such recombinant receptor has the same properties as the cellular receptor, as far as its recognizing activity is concerned.

Monoclonal antibodies of the invention can be obtained against various forms of the receptor, including the complete receptor, a particular domain or a peptide characteristic of the aminoacid sequence of the receptor represented in figure 3.

Monoclonal antibodies of the invention can for example be prepared against the soluble form of the receptor. A hydrosoluble polypeptide corresponding to the soluble form of the INF-R is described on figure 2. According to the present invention, a soluble form of the IFN-R corresponds to a peptide or a polypeptide, capable of circulating in the body.

Other monoclonal antibodies according to the invention can also be prepared against a peptide comprised in the extracellular domain of the receptor as described on figure 2. An advantageous peptide corresponds for instance to the aminoacid sequence comprised between aminoacid 1 and aminoacid 229. According to another embodiment of the invention, the antibodies can be prepared against a polypeptide modified by substitution of one or more amino acids, provided that antibodies directed against the non modified extracellular domain of the IFN-R, recognize the modified polypeptide or peptide.

Preferred monoclonal antibodies according to the invention are those which are of the IgG1 type.

Among the antibodies of the invention, an antibody which has the capacity of inhibiting the binding of the type I-IFN to its receptor is preferably characterized in that it inhibits the in vitro binding of human type IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0.5 to 2 $\mu\text{g/ml}$.

The inventors have shown that the high affinity binding capacity of a monoclonal antibody is not sufficient to ensure that this antibody will be able to inhibit the binding activity of the human type I-IFN to the IFN-R. Nevertheless the high affinity binding capacity of the monoclonal antibody is necessary to investigate further the ability of the antibody to inhibit the binding of the type I-IFN to its cellular receptor.

Another monoclonal antibody is characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

According to another embodiment a monoclonal antibody is also characterized in that it neutralizes in vitro the antiproliferative activity of human type IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

A particular group of monoclonal antibodies according to the invention is characterized in that it

neutralizes the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 50 $\mu\text{g/ml}$, preferably 1 to 20 $\mu\text{g/ml}$, for a concentration of type I-IFN in the range of 1 to 1000 units with reference to the international standard MRC 69/19.

Advantageously, the monoclonal antibody according to the invention is such that these antibodies do not bind to the human receptor for IFN gamma.

One particular antibody satisfying the requirements of the invention, is such as it directed against an epitope on the amino-acid sequence comprised between amino-acid 27 and amino-acid 427 of the extracellular domain of the human IFN-R as represented on figure 2.

One particularly interesting monoclonal antibody according to the invention is the antibody designated 64G12 under n° 92022605 which has been deposited at the ECACC (European Collection of Animal Cell Cultures Porton Down Salisbury, Wiltshire SP4 056, United Kingdom) on February 26, 1992.

These antibodies may be prepared by conventional methods involving the preparation of hybridoma cells by the fusion of myeloma cells and spleen cells of an animal immunized beforehand with the peptide antigen, on the conditions such that the antigen against which the antibodies are formed is constituted by the extracellular domain of IFN-R or any polypeptide or peptide of this domain.

The hybridomas are constructed according to the protocole of Kohler and Milstein (Nature, 1974, 256: 495-497). For example the hybridomas are derived from

the fusion of the spleen cells above described with NS1 mouse (BalbC) HGPRT⁻ as myeloma cell.

A second procedure for the production of monoclonal antibodies according to the invention, consists in carrying out the fusion between B-cells of blood immortalized with the Epstein/Barr virus and human B lymphocytes placed beforehand in contact with the extracellular domain or a fragment thereof of the IFN-R, against which it is decided to form monoclonal antibodies. B-cells placed in contact beforehand with the extracellular domain of IFN-R or fragment thereof against which it is decided to form monoclonal antibodies, may be obtained by in vitro culture contacted with the antigens, the recovery of the B-cells coated with these antigens being preceded by one or several cycles of stimulation.

The invention thus concerns human antibodies as obtained by carrying out the above procedure, having the above defined properties.

The invention also aims at providing a monoclonal antibody characterized in that the variable or complementary determining regions of its heavy and/or light chains are grafted on the framework and/or constant regions of a human antibody.

The invention further provides a composition having antagonist properties for the biological properties of the human type I-IFN, characterized in that it comprises monoclonal antibodies as defined above.

Accordingly the invention provides a pharmaceutical composition characterized in that it comprises monoclonal antibodies as defined above, together with an appropriate pharmaceutical vehicle.

The invention also concerns the use of a monoclonal antibody as defined above, for the manufacture of a drug for the treatment or profilaxis of a pathological state or symptoms associated with overproduction of type-I-IFN.

According to a first example, the antibodies can be used in a pharmaceutical composition, for the treatment of allograft rejection.

According to another example, antibodies of the invention are used as active principle in a pharmaceutical composition for the treatment of autoimmune and inflammatory diseases. Such diseases include systemic lupus erythematosus, type 1 diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, acquired immunodeficiency syndrome (AIDS), and severe combined immunodeficiency disease.

Treatment of acute virus diseases can also be performed with the antibodies of the invention. As example upper respiratory tract infections, chronic virus infections such as those due to measles virus, can be performed.

The antibodies of the invention can also be used for the in vitro diagnosis of the presence of the human type I-IFN receptor or cells.

Further details and additional information will arise from the description from the description of the examples and from the figures.

FIGURES

- Figure 1 : binding of ^{125}I -labelled monoclonal antibodies 34F10 and 64G12 to :

- A : Daudi cells
- B : Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was mesured by incubation with a 100 fold exces of cold antibodies and substracted from total counts.

- Figure 2 : nucleotide and corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthetized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in figure 2.

- Figure 3 : nucleotide and corresponding amino-acid sequence of the human IFN-R.

E X A M P L E S**EXAMPLE 1 :**Synthesis of the soluble receptorsSynthesis in E.coli

A fragment of DNA containing the sequence coding for the extracellular domain (amino acids 27 to 427) of the human INF-R (figure 2), in which an extra-sequence coding for 5 histidyl residues was introduced just before the termination codon, was cloned in the expression vectors pKK233-2. This fragment was produced by the Polymerase Chain Reaction (PCR) and the resulting plasmids were sequenced to confirm both in-frame insertion with the Shine-Dalgarno sequence and the appropriate sequence coding for the receptor.

The poly-histidyl tail introduced into the recombinant protein enables it to be purified rapidly by affinity chromatography on a chelated nickel support (NTA column) as described previously (Hochuli E. et al, Bio/technology, 1988, 1321-1325).

The plasmid was introduced into the E.coli strain, JM105, and protein synthesis induced by addition of IPTG to the culture medium (pKK233-2, tac promoter).

Proteins were extracted from the bacterial pellet and the soluble receptor purified to homogeneity by affinity chromatography as described hereafter. This procedure yielded a protein that migrates as 2 bands around 50 kDa under reducing conditions and three bands under non-reducing conditions. The maximum concentration of the protein obtained by different procedures was approximately 20µg/ml.

The N-terminal sequence of the two proteins detected by gel electrophoresis has shown that both proteins are the expected fragment of the receptor.

Synthesis and purification of an unglycosylated soluble receptor :

Bacterial culture (250ml)

|

IPTG induction 3h

|

cell pellet

6M Guanidine hydrochloride pH8

|

centrifugation

|

NTA column:

Washes pH 8 urea 8M

pH 6,3 urea 8M

pH 5.9 urea 8M

|

Elution pH 4 urea 8M

|

refolding dilution, dialysis

|

against Tris 0,1 M pH9

dialysis PBS

Using the same PCR approach, we also constructed an expression vector coding for the IFN-R amino acid sequence 1-427, with an additional 5-histidyl residues at the C-terminus, inserted in expression vector pXMT-3. The exact nucleotide sequence of the insert was also confirmed.

The resulting plasmid was introduced by electroporation into Cos7 cells for transient expression and the recombinant protein was purified to homogeneity by affinity chromatography followed by ion exchange chromatography on mono-Q (Pharmacia) as described hereafter.

Purification of the soluble IFN-R from Cos7 cells

preparative electroporation of
cos cells

| 18 h
|

serum free medium
|

supernatants taken after 48h, 72h, 96h
|

concentration
|

NTA column
|

Wash PBS

elution 0.1 M NaOAc pH 5.5
|

neutralization

concentration, 30 000 cut off
|

Mono Q (0-0.5 M Na Cl)

This purification yielded to a 76 kDa protein whose N-terminal sequence corresponds to the predicted receptor sequence with some heterogeneity in the processing of the leader sequence.

EXAMPLE 2 :

Production of monoclonal antibodies against the interferon type I receptor

1) Production of the monoclonal antibodies

Mice were immunized by injection of recombinant soluble interferon (r sIFN-R) purified from E.coli or from a culture supernatant of Cos7 cells. Initially mice were injected both intraperitoneally and subcutaneously with the purified protein in complete Freund's adjuvant. Subsequently mice were injected once a week intraperitoneally with the purified proteins diluted in buffered saline solution. Ten micrograms of recombinant proteins were injected each time.

After the fourth injection, blood was collected and the presence of specific serum antibodies were tested by both ELISA and Western blot against the recombinant receptor. The strongest responders were then boosted with a total of 10 μ g of antigen half of which was injected intravenously and half intraperitoneally.

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT⁻ myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35:1-32, 1980). Briefly, 5×10^7 spleen cells were fused to 3×10^7 myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as 20×10^7 spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method :

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.05% Tween 20,

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% Tween 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

3) Identification of reactivity to the natural interferon type I receptor

The reactivity of the monoclonal antibodies (mAbs) recognizing the recombinant sIFN-R was tested against the natural class I receptor expressed at the surface of Daudi cells, by membrane immunofluorescence. Briefly, 5×10^5 Daudi cells were incubated in 100 μ l of culture supernatant of chosen hybridomas for 30 min at 4°C. The cells were then washed 4 times in RPMI medium containing 1% BSA and further incubated with a diluted FITC labelled goat anti-mouse F(ab')₂ for 30 min at 4°C. The cells were finally analyzed by flow cytometry after washing. One of the 35 tested antibodies produced against the E.coli recombinant receptor and 5 of the 6 tested antibodies produced against the COS recombinant receptor were found to recognize the natural receptor on the Daudi cells.

Cloning of these hybridomas was then performed by limiting dilution. The isotype of these mAbs was determined by an ELISA method using isotype specific antibodies. All 6 mAbs were found to be IgG1 with kappa light chains. A summary of the reactivity of these 6 mAbs is given in Table 1.

Monoclonal antibodies were purified from culture supernatants by protein G chromatography.

Table 1 :**Reactivity of the anti IFN-R monoclonal antibodies**

	Reactivity against the recombinant receptor				Reactivity against * the cellular receptor
	E.COLI		COS		immunofluorescence
	ELISA	Western	ELISA	Western	
34F10	+	+	+	+	+
64G12	+	+	+	+	+
63F6 64G2 64D10 65D8	-	-	+	+ weak	+

* measured on Daudi cells

EXAMPLE 3 :**Inhibition of the binding of interferon to human cell lines**

Inhibition of interferon binding to human cells was assayed as follows. 10^6 cells were preincubated at 4°C for 30 min with various dilutions of hybridoma culture supernatants or purified mAbs or with medium alone. ^{125}I -labelled IFN alpha 8 or alpha 2 was added at the concentration of 100pM and cells incubated for a further 2 hours at 4°C. These incubations were performed in RPMI medium containing 20mM HEPES pH 7.4 and 10% foetal calf serum (FCS). The cells were finally washed 4 times with RPMI - 1% FCS and counted to determine bound radioactivity.

The mAb secreted by the hybridoma line 64G12 (latter named mAb 64G12) was shown in this assay to inhibit the binding of labelled IFN to the cells in a dose-dependent manner. 50% inhibition of binding to the Daudi cells (Burkitt lymphoma cell line ; Klein et al., Cancer Research, 28:1300-1310, 1968) was obtained at a mAb concentration of 0.4 μ g/ml. The same inhibition was obtained with K562 cells (chronic myelogenous leukemia, Lozzio and Lozzio, Cell, 45:321-334, 1975) but 50% inhibition was obtained at 11 μ g/ml for HL60 cells (Promyelocytic leukemia, Collins S.J. et al., Nature, 270:347-349, 1977) and 60 μ g/ml for Ly28 cells (Klein G. et al. Int. J. Cancer, 10:44-57, 1972).

Table 2 :

The inhibition of binding of labelled IFN alpha 2 to various cell lines by mAb64G12

Cell lines	Concentration of mAB which gives 50% inhibition of binding
Daudi K562	0,4 μ g/ml
HL60	11 μ g/ml
Ly28	60 μ g/ml

The difference in the mAb concentration at which 50% inhibition of binding of IFN is obtained has been investigated by direct binding of ¹²⁵I-labelled mABs 64G12 and 34F10 to the same cell lines and Scatchard

plot analysis of the results. In the concentration range of 0.1 to 1.5 $\mu\text{g/ml}$, a high affinity binding of the mAb 34F10 ($\approx 10\text{nM}$) was seen on all cell lines whereas a high affinity binding of mAb 64G12 was only detected on Daudi and K562 cells (Figure 1).

EXAMPLE 4 :

Inhibition of the function of type I interferon

Functional inhibition of type I interferon by the purified mAb 64G12 was demonstrated in an antiviral assay on Daudi cells using either recombinant IFN alpha 2, IFN beta and IFN omega, or purified Namalwa and leucocyte interferons, and in an antiproliferative assay with recombinant IFN alpha 2.

* Antiviral activity

An antiviral assay on Daudi cells was performed as described (M. Dron and M.G. Tovey, J. Gen. Virol. 64:2641-2647, 1983). Cells ($0.5 \times 10^6/\text{ml}$) were incubated for 24 hours in the presence of interferon and antibodies. 10^6 cells in 1 ml were then infected for 1 hour at 37°C with Vesicular stomatitis virus (VSV) then washed 3 times, resuspended in culture medium and incubated for 18 hours at 37°C . Cells were then lysed by freeze-thawing and virus replication measured by titration of the supernatants on L929 cells. A dose-dependent inhibition of the antiviral activity of the various subtypes of type I IFN was demonstrated for the purified mAb 64G12.

For the antiviral assay with the Wish cells, cells were incubated for 24 hours with various concentrations of interferons in the presence of the mAbs prior to challenge with VSV. In this assay, the mAb 64G12 was demonstrated to block completely the antiviral activity of Leukocyte IFN (50U/ml), recombinant IFN alpha 2 (50U/ml) and interferon from the sera of AIDS patients (50, 75 and 150U/ml).

* antiproliferative activity

For the antiproliferative assay, Daudi cells were seeded at a concentration of 10^5 cells per ml in a 96 well plate in the presence of interferon and purified inhibitory or control antibody. Cells were then counted after 24, 48 and 72 hours with a Coulter counter and checked for viability by trypan blue exclusion. Purified mAb 64G12 demonstrated a dose-dependent inhibition of the antiproliferative activity of interferon alpha 2.

C L A I M S

1. Monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :
 - it recognizes the extracellular domain of the human IFN-R, and
 - it has a neutralizing capacity against the biological properties of the human type I-IFN.
2. Monoclonal antibody directed against the human type I IFN-R according to claim 1, characterized by its capacity to inhibit the binding of a human pathological type I-IFN, to the IFN-R.
3. Monoclonal antibody according to claim 1 or 2, which is obtainable from a hybridoma cell prepared by fusion of a myeloma cell with spleen cells from an animal previously immunized with the soluble form of the human IFN-R.
4. Monoclonal antibody according to anyone of claims 1, 2 or 3, characterized in that it recognizes an epitope on a soluble form of the human cellular IFN-R or of a recombinant IFN-R.
5. Monoclonal antibody according to anyone of claims 1 to 4, characterized in that it inhibits in vitro the binding of human type I-IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0,5 to 2 $\mu\text{g/ml}$.
6. Monoclonal antibody according to anyone of claims 1 to 5, characterized in that it neutralizes in vitro the antiproliferative activity of the human type I-IFN, on cells highly responsive to this human type I-IFN,

for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

7. Monoclonal antibody according to anyone of claims 1 to 6, characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells poorly responsive to this human type I-IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

8. Monoclonal antibody according to anyone of claims 1 to 7, characterized in that it does not bind to the human receptor of the IFN gamma.

9. Monoclonal antibody according to anyone of claims 1 to 8, characterized in that it recognizes an epitope on the aminoacid sequence 27 to 427 of the human IFN-R.

10. Monoclonal antibody according to anyone of claims 1 to 9, characterized in that it neutralizes in vitro the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

11. Monoclonal antibody according to anyone of claims 1 to 10, characterized in that it neutralizes in vitro the antiviral activity of the human class I-IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

12. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is the 64G12 antibody, deposited at the ECACC on February 26, 1992 under n° 92022605.

13. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a humanized antibody, for instance characterized in that the variable or complementary determining regions of its

heavy and light chains are grafted on the framework and constant regions of a human antibody.

14. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a human antibody.

15. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is an IgG1 type antibody.

16. Hybridoma cell, characterized in that it produces monoclonal antibodies according to claims 1 to 13.

17. Composition having antagonist properties to the type I-IFN, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 16.

18. Pharmaceutical composition, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 17, together with an appropriate pharmaceutical vehicle.

19. Use of a monoclonal antibody according to anyone of claims 1 to 17, for the manufacture of a drug for the treatment or prophylaxis of a pathological state associated with proliferative cell activity and/or viral cell infection.

20. Process for the selection of a monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN, to the IFN-R, characterized by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies according to anyone of claims 1 to 15 or a hybridoma culture supernatant containing monoclonal antibodies, with human cells susceptible of harboring IFN-R ;
- adding labelled human type I-IFN in a determined concentration, to the above preincubating medium ;

- incubating the medium containing the human cells, monoclonal antibodies and labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the type I-IFN, by counting the amount of attached labelled type I-IFN.

21. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiproliferative activities of the type I-IFN, on human cells characterized by the steps of :

- allowing cells to grow in the presence of human type I-IFN and in the presence of a determined concentration of monoclonal antibodies according to anyone of claims 1 to 15 ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the type I-IFN.

22. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiviral activities of the natural, non pathological or pathological type I-IFN on human cells, characterized by the steps of :

- incubating cells with type I-IFN and monoclonal antibodies according to anyone of claims 1 to 15, in determined concentrations, for a time sufficient to allow the formation of a complex

between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the above incubated cells with a determined concentration of a virus ;
- washing the cells ;
- resuspending the cells in culture medium ;
- incubating for a time sufficient to allow the replication of the virus ;
- lysing the cells and ;
- measuring the virus replication or measuring the inhibition of the cytopathic effect.

A B R I D G M E N TMONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The invention relates to a monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

It further concerns their use for the diagnosis.

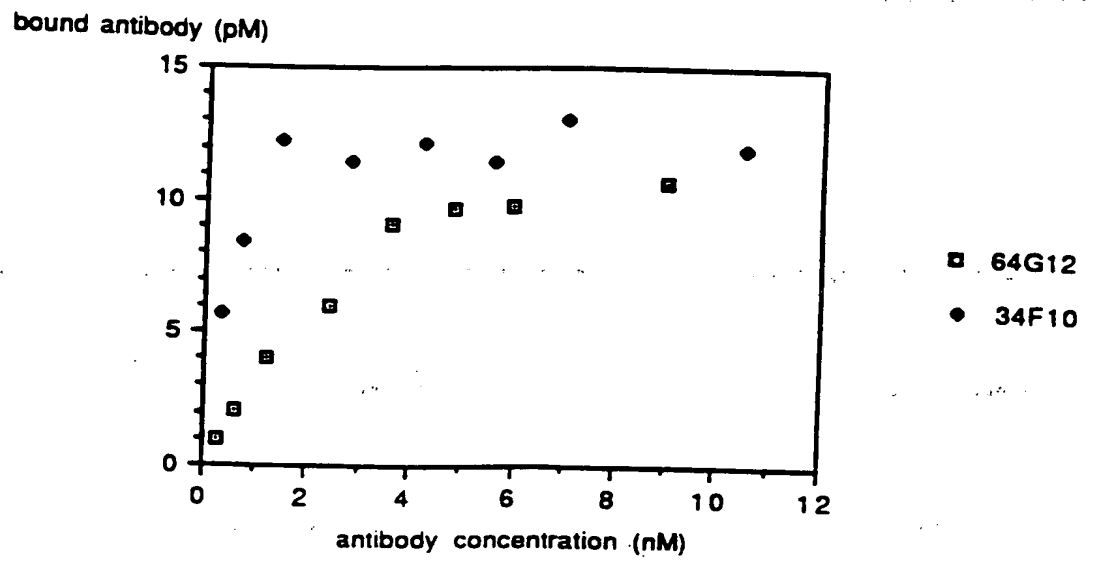
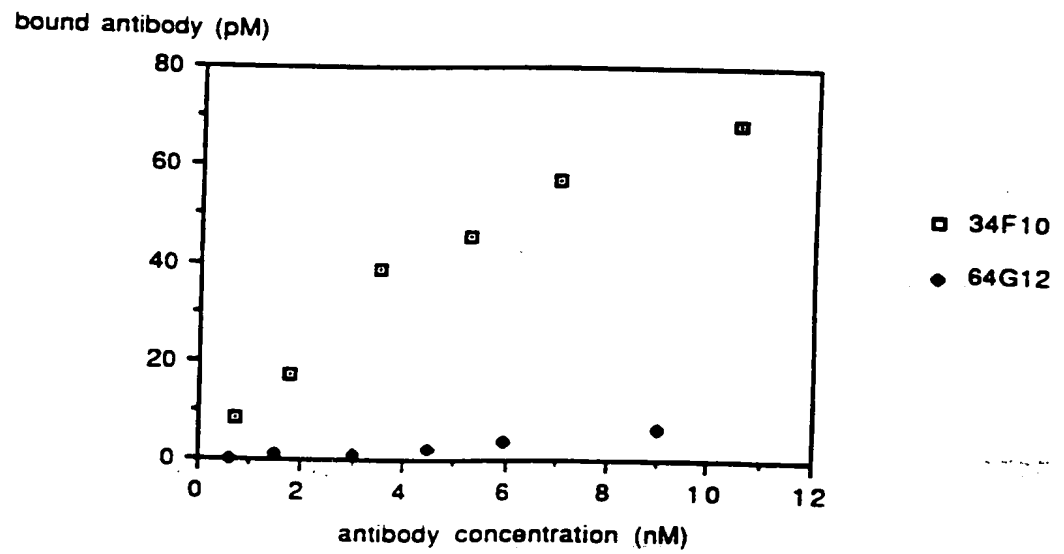
A**B**

FIGURE 1

CTGCAGGCATCTGCGCGGCTCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 2A

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn MET Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu

AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr MET Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA TCAGGTACC
Asn Thr Ser Lys

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FIGURE 2B

CTGCAGGGATCTCGGCGGGCTCCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 3A

ATT GAA AAT ATA AGC ACA ATT GCT ACA GTA GAA GAA ACT AAT CAA ACT GAT GAA
 Ile Glu Asn Ile Ser Thr Ile Ala Thr Val Glu Glu Thr Asn Gln Thr Asp Glu

GAT CAT AAA AAA TAC AGT TCC CAA ACT AGC CAA GAT TCA GGA AAT TAT TCT AAT
 Asp His Lys Lys Tyr Ser Ser Gln Thr Ser Gln Asp Ser Gly Asn Tyr Ser Asn

GAA GAT GAA AGC GAA AGT AAA ACA AGT GAA GAA CTA CAG CAG GAC TTT GTA TCA
 Glu Asp Glu Ser Glu Ser Lys Thr Ser Glu Glu Leu Gln Gln Asp Phe Val

CCAGAAATGAACTGTGTCAAGTATAAGGTTTTTCAGCAGGAGTTACACTGCTACC

1697

FIGURE 3C

ANNEX 5

Translation of the letter dated July 13, 2001, that was sent to the inventors

Annex 5 :
**Translation of the letter that was sent to the inventors
together with a copy of the supplemental declaration**

Re: US Patent No. US 5,919,453 of July 6, 1999
Divisional Application N° 09/240,675 filed on February 2, 1999
in the name of MEDISUP INTERNATIONAL N.V.
Inventors: P. BENOIT, F. MEYER, D. MAGUIRE, I. PLAVEC, M.G. TOVEY

Dear Sir :

As an inventor named in the above-cited US patent, you have signed, on November 11, 1994, a Declaration giving power to our Associates of the firm Foley & Lardner, so that they represent you before the American Patent Office. A copy of this Declaration is enclosed.

Within the context of a divisional patent application, pertaining to specific peptides recognized by the monoclonal antibody 64G12, the American Examiner responsible for this file considers that this declaration is defective because certain handwritten corrections have been added thereto without being dated and initialled. Therefore, he asks us to provide a new declaration.

You will hence find enclosed a copy of the text initially filed as well as a copy of the claims of the divisional application.

You will find also a copy of a supplemental declaration, that we ask you to kindly send back to us after you have dated and signed it. We draw your attention to the content of the sixth paragraph of this declaration, that specifies that you have read and understood the specification and all the enclosed claims.

Should you have any question concerning this matter, please do not hesitate to contact us. Moreover, should you decide not to sign this declaration, we thank you to kindly inform us.

Sincerely yours,

Encl.: -declaration of 11/11/94
- text initially filed and claims of the divisional application
- supplemental declaration

VERIFICATION OF TRANSLATION

I, Julia ANDRAL-ZIURYS, working at ERNEST GUTMANN-YVES PLASSERAUD S.A., 3 rue Chauveau-Lagarde, 75008 Paris (France),

declare that I am conversant with the French and English languages and that to the best of my knowledge and belief the following (Annex 5) is a true translation of our registered letter that was sent to the last known address of each of the inventors in Annex 4.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application or any patent issuing thereon.

Paris, November 22, 2001

Signature:

A handwritten signature in black ink, appearing to read "Julia Andral-Ziurys", written in a cursive style.

Julia ANDRAL-ZIURYS

22/11/01

ANNEX 6

Declaration dated and signed by Mr. TOVEY on July 23, 2001.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR,
WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

(Attorney Docket No. 017283/0123)

the specification of which (check one)

☐ Is attached hereto.

☒ Was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
92400902.0	European	31/March/1992	Yes	NO

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

STEPHEN A. BENT	Reg. No. 29,768
DAVID A. BLUMENTHAL	Reg. No. 26,257
BETH A. BURROUS	Reg. No. 35,087
ALAN I. CANTOR	Reg. No. 28,163
WILLIAM T. ELLIS	Reg. No. 26,874
JOHN J. FELDHAUS	Reg. No. 28,822
MICHAEL D. KAMINSKI	Reg. No. 32,904
LYLE K. KIMMS	Reg. No. 34,079
KENNETH E. KROSIN	Reg. No. 25,735

JOHNNY A. KUMAR	Reg. No. 34,649
JACK LAHR	Reg. No. 19,621
GLENN LAW	Reg. No. 34,371
PETER G. MACK	Reg. No. 26,001
STEPHEN B. MAEBIUS	Reg. No. 35,264
BRIAN J. MC NAMARA	Reg. No. 32,789
SYBIL MELOY	Reg. No. 22,749
RICHARD C. PEET	Reg. No. 35,792
GEORGE E. QUILLIN	Reg. No. 32,792
ANDREW E. RAWLINS	Reg. No. 34,702
BERNHARD D. SAXE	Reg. No. 28,665
CHARLES F. SCHILL	Reg. No. 27,590
RICHARD L. SCHWAAB	Reg. No. 25,479
MICHELE M. SIMKIN	Reg. No. 34,717
HAROLD C. WEGNER	Reg. No. 25,258

to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

I request that all correspondence be directed to:

Bernhard D. Saxe
Foley & Lardner
At Washington Harbour
3000 K Street, NW, Suite 500
Washington, DC 2000

Telephone: 202-672-5472
Facsimile: 202-672-5399

I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Name of first inventor	<i>Patrick BENOIT</i>
Residence	<i>Paris, France</i>
Citizenship	<i>FRANCE</i>
Post Office Address	<i>24, rue Jonquoy, F-75014 Paris, France</i>
Inventor's signature	
Date	

Name of second inventor	<i>Francois MEYER</i>
Residence	<i>Paris, France</i>
Citizenship	<i>LUXEMBOURG</i>
Post Office Address	<i>3 Place du Panthéon, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of third inventor	<i>Deborah MAGUIRE</i>
Residence	<i>Paris, France</i>
Citizenship	<i>AUSTRALIA</i>
Post Office Address	<i>24, rue Maître-Albert, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of fourth inventor	<i>Ivan PLAVEC</i>
Residence	<i>Fresnes, France</i>
Citizenship	<i>CROATIA</i>
Post Office Address	<i>1, allée du Capitaine-Dupont F-92260 Fresnes, France</i>
Inventor's signature	
Date	
Name of fifth inventor	<i>Michael G. TOVEY</i>
Residence	<i>Paris, France</i>
Citizenship	<i>GREAT BRITAIN</i>
Post Office Address	<i>7, rue Lagrange, F-75005 Paris, France</i>
Inventor's signature	<i>Michael Tovey</i>
Date	<i>23 July 2001</i>

172831-

ANNEX 7

Copy of our letter of August 10, 2001, to Mr. PLAVEC, at his new address in the U.S.



ERNEST GUTMANN - YVES PLASSERAUD S.A.
SOCIETE DE CONSEILS EN PROPRIETE INDUSTRIELLE

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VIA FACSIMILE
+ CONFIRMATION BY REGISTERED MAIL

Mr. Ivan PLAVEC
Senior Director, Functional Genomics
Bioseek, Inc.
863-C Mitten Road
Burlingame, CA 94010
U.S.A.

VOTRE REFERENCE :

NOTRE REFERENCE :

LE

B1608ACA – AD/VMA/MNH
August 10, 2001

Re: **"MONOCLONAL ANTIBODY ALPHA IFN"**
US Patent No. 5,919,453 of July 6, 1999
Divisional Application No. 09/240,675 filed on February 02, 1999
in the name of: MEDISUP INTERNATIONAL N.V.
Inventors: BENOIT, MEYER, MAGUIRE, PLAVEC, TOVEY

BREVETS

Ernest GUTMANN, cpl *
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Jeanne VAILLANT, cpl *
Lionel BIROTHEAU
Véronique MARCADÉ
Denis BOURGAREL
Carole SELLIN⁽²⁾

**MARQUES, DESSINS
ET MODÈLES**

Yves PLASSERAUD, cpl *
Martine DEHAUT, cpl *
Virginie ZANCAN, cpl *
Nathalie PACAUD
Benjamin FONTAINE⁽³⁾
Christophe PELÈSE

**DOCUMENTATION
ET VEILLE TECHNOLOGIQUE**
Jean-Charles THEODET

Dear Mr. Plavec:

Thank you for your facsimile of August 6, 2001, in reply to our phone message.

We are the representatives of MEDISUP INTERNATIONAL N.V. regarding patent applications for the above mentioned invention, of which you are one of the inventors.

Please find enclosed our letter of July 13, 2001 that we sent to your former address, transmitting to you a new "Declaration and Power of Attorney" in substitution of the declaration you signed on November 11, 1994. As you can read from our letter, this new oath is required by the United States Patent and Trademark Office. So, please return to us this new declaration signed and dated as soon as possible.

Please also complete your personal address by hand and date and initial after this completion.

Should you have any questions concerning this matter, please do not hesitate to contact us.

Yours sincerely,

Véronique MARCADÉ

Julia ANDRAL-ZIURYS

Encl. : our letter of 13.07.01 and enclosures

*mandataire agréé OEB/EPO
*US patent attorney
européen en marques
OHM/OHIM

⁽¹⁾Agence de Lyon
⁽²⁾Agence d'Alicante

SOCIETE ANONYME
AU CAPITAL DE 3 000 000 F
RCS PARIS B 332 417 500
APE 741 A

Acquisition et défense des droits, stratégie de protection, liberté d'exploitation
et recherches de disponibilité, oppositions, consultations, contrats et audits

ANNEX 8

Declaration signed and dated by Mr. PLAVEC on August 27, 2001.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR,
WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

(Attorney Docket No. 017283/0123)

the specification of which (check one)

☐ Is attached hereto.

☒ Was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
92400902.0	European	31/March/1992	Yes	NO

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

STEPHEN A. BENT	Reg. No. 29,768
DAVID A. BLUMENTHAL	Reg. No. 26,257
BETH A. BURROUS	Reg. No. 35,087
ALAN I. CANTOR	Reg. No. 28,163
WILLIAM T. ELLIS	Reg. No. 26,874
JOHN J. FELDHAUS	Reg. No. 28,822
MICHAEL D. KAMINSKI	Reg. No. 32,904
LYLE K. KIMMS	Reg. No. 34,079
KENNETH E. KROSIN	Reg. No. 25,735

JOHNNY A. KUMAR	Reg. No. 34,649
JACK LAHR	Reg. No. 19,621
GLENN LAW	Reg. No. 34,371
PETER G. MACK	Reg. No. 26,001
STEPHEN B. MAEBIUS	Reg. No. 35,264
BRIAN J. MC NAMARA	Reg. No. 32,789
SYBIL MELOY	Reg. No. 22,749
RICHARD C. PEET	Reg. No. 35,792
GEORGE E. QUILLIN	Reg. No. 32,792
ANDREW E. RAWLINS	Reg. No. 34,702
BERNHARD D. SAXE	Reg. No. 28,665
CHARLES F. SCHILL	Reg. No. 27,590
RICHARD L. SCHWAAB	Reg. No. 25,479
MICHELE M. SIMKIN	Reg. No. 34,717
HAROLD C. WEGNER	Reg. No. 25,258

to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

I request that all correspondence be directed to:

Bernhard D. Saxe
Foley & Lardner
At Washington Harbour
3000 K Street, NW, Suite 500
Washington, DC 2000

Telephone: 202-672-5472
Facsimile: 202-672-5399

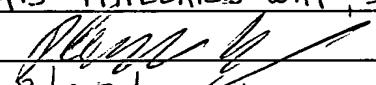
I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Name of first inventor	<u>Patrick BENOIT</u>
Residence	<u>Paris, France</u>
Citizenship	<u>FRANCE</u>
Post Office Address	<u>24, rue Jonquoy, F-75014 Paris, France</u>
Inventor's signature	<u></u>
Date	<u></u>

Name of second inventor Francois MEYER
Residence Paris, France
Citizenship LUXEMBOURG
Post Office Address 14, square Adanson, F-75005 Paris, France
Inventor's signature _____
Date _____

Name of third inventor Deborah MAGUIRE
Residence Paris, France
Citizenship AUSTRALIA
Post Office Address 24, rue Maitre-Albert, F-75005 Paris, France
Inventor's signature _____
Date _____

Name of fourth inventor Ivan PLAVEC
Residence SUNNYVALE, CA, USA
Citizenship CROATIA
Post Office Address 1415 MALLARD WAY, SUNNYVALE CA 94087, USA
Inventor's signature 
Date 8/27/2001

Name of fifth inventor Michael G. TOVEY
Residence Paris, France
Citizenship GREAT BRITAIN
Post Office Address 6, rue des Quatrefages, F-75005 Paris, France
Inventor's signature _____
Date _____

ANNEX 9

Acknowledgement of receipt n° 6156 2081 5FR of our registered mail of July 16, 2001 to Mr. MEYER and collected on July 18, 2001.

Ann x 9 : Acknowledgement of receipt n° 6156 2081 5FR of our registered mail of July 16, 2001 to Mr. MEYER and collected on July 18, 2001.

LA POSTE 

RA 6156 2081 5FR
Saculuna C. LEBERACA

**AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDÉ**

Présenté le : 17/7/01

Distribué le :

Signature du destinataire : 



RETOUR A :
GUY-HENRI MASSERAUD
3 Rue Chauveau-Lagarde
75008 PARIS

AVIS DE RÉCEPTION **AR**

SIREN 356 000 000 RCS NANTERRE

ANNEX 10

Translation from French into English of Annex 9.

Annex 10: acknowledgment of receipt

Post reference → RA 6156 2081 5FR
Our reference →
Presented on (date) →
Delivered on (date) →
Addressee's signature →

LA POSTE

Présenté le : 11/11/18
Distribué le :
Signature du destinataire :

AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDÉ

Stamp showing
that the mail was
collected on July 7, 2001

RETURN TO
M. GUTHRIE - DASSEREAU
13 Rue Chauveau-Lagarde
75008 PARIS

ACKNOWLEDGMENT
of Receipt

Return to

acknowledgment of receipt of your
registered mail

AR

SIREN 356 000 000 RCS NANTERRE

VERIFICATION OF TRANSLATION

I, Julia ANDRAL-ZIURYS, working at ERNEST GUTMANN-YVES PLASSERAUD S.A., 3 rue Chauveau-Lagarde, 75008 Paris (France),

declare that I am conversant with the French and English languages and that to the best of my knowledge and belief the following (Annex 10) is a true translation of the Acknowledgement of receipt n° 6156 2081 5FR (Annex 9) of our registered mail of July 16, 2001 to Mr. MEYER and collected on July 18, 2001.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application or any patent issuing thereon.

Paris, November 22, 2001

Signature:




Julia ANDRAL-ZIURYS

22/11/01

ANNEX 11

Acknowledgement of receipt n° 6156 2268 8FR of the 2nd sending of September 4, 2001, of the same package to Mr. MEYER with the same enclosures, and collected on September 6, 2001.

Annex 11 : Acknowledgement of receipt n° 6156 2268 8FR of the 2nd sending of September 4, 2001, of the same package to Mr. MEYER with the same enclosures, and collected September 6, 2001.

LA POSTE 

RA 6156 2268 8FR
6156 8FR / Jaz / VMA / MNH

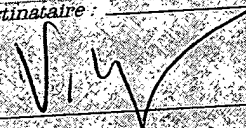
Annex 11

**AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDÉ**

19 H
6-9
2001
PARIS 5^e

Présenté le : 6/9/01

Distribué le :

Signature du destinataire : 

M. François Meyer
3 Place du Panthéon
75005 Paris

RETOUR A :
Gutmann Plassard
3 Rue Châteauneuf Legendre
75008 Paris


SIREN 356 000 000 ROS NANTERRE

AVIS DE RÉCEPTION

ANNEX 12

Acknowledgement of receipt n° 6156 2426 4FR of the 3rd sending of October 5, 2001, of the same package to Mr. MEYER with the same enclosures, collected on October 9, 2001.

Annex 12 : Acknowledgement of receipt n° 6156 2426 4FR of the 3rd sending of October 5, 2001, of the same package to Mr. MEYER with the same enclosures, collected on October 9, 2001.

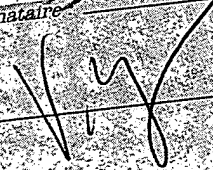
LA POSTE 

**AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDE**

RA 6156 2426 4FR
Jan 10/01 - 16880000 → VMA

Présenté le : 10/01

Distribué le : 10/01

Signature du destinataire: 

RETOUR A:
M. François Meyer
3 Place du Panthéon
75005 Paris
Gutmann - Plassard
3 rue Chauveau Lagarde
75008 Paris

SIREN 368 000 000 RCS NANTERRE

AR
AVIS DE RÉCEPTION

ANNEX 13

Declaration dated and signed by Mr. MEYER on November 5, 2001.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR,
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(Attorney Docket No. 017283/0123)

the specification of which (check one)

☐ Is attached hereto.

☒ Was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

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92400902.0	European	31/March/1992	Yes	NO

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

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U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

STEPHEN A. BENT	Reg. No. 29,768
DAVID A. BLUMENTHAL	Reg. No. 26,257
BETH A. BURROUS	Reg. No. 35,087
ALAN I. CANTOR	Reg. No. 28,163
WILLIAM T. ELLIS	Reg. No. 26,874
JOHN J. FELDHAUS	Reg. No. 28,822
MICHAEL D. KAMINSKI	Reg. No. 32,904
LYLE K. KIMMS	Reg. No. 34,079
KENNETH E. KROSIN	Reg. No. 25,735

JOHNNY A. KUMAR	Reg. No. 34,649
JACK LAHR	Reg. No. 19,621
GLENN LAW	Reg. No. 34,371
PETER G. MACK	Reg. No. 26,001
STEPHEN B. MAEBIUS	Reg. No. 35,264
BRIAN J. MC NAMARA	Reg. No. 32,789
SYBIL MELOY	Reg. No. 22,749
RICHARD C. PEET	Reg. No. 35,792
GEORGE E. QUILLIN	Reg. No. 32,792
ANDREW E. RAWLINS	Reg. No. 34,702
BERNHARD D. SAXE	Reg. No. 28,665
CHARLES F. SCHILL	Reg. No. 27,590
RICHARD L. SCHWAAB	Reg. No. 25,479
MICHELE M. SIMKIN	Reg. No. 34,717
HAROLD C. WEGNER	Reg. No. 25,258

to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

I request that all correspondence be directed to:

Bernhard D. Saxe
Foley & Lardner
At Washington Harbour
3000 K Street, NW, Suite 500
Washington, DC 2000

Telephone: 202-672-5472
Facsimile: 202-672-5399

I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Name of first inventor	<i>Patrick BENOIT</i>
Residence	<i>Paris, France</i>
Citizenship	<i>FRANCE</i>
Post Office Address	<i>24, rue Jonquoy, F-75014 Paris, France</i>
Inventor's signature	
Date	

Name of second inventor	<i>Francois MEYER</i>
Residence	<i>Paris, France</i>
Citizenship	<i>LUXEMBOURG</i>
Post Office Address	<i>3 Place du Panthéon, F-75005 Paris, France</i>
Inventor's signature	<i>X [Signature]</i>
Date	<i>X 5th November 2001</i>
Name of third inventor	<i>Deborah MAGUIRE</i>
Residence	<i>Paris, France</i>
Citizenship	<i>AUSTRALIA</i>
Post Office Address	<i>24, rue Maître-Albert, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of fourth inventor	<i>Ivan PLAVEC</i>
Residence	<i>Fresnes, France</i>
Citizenship	<i>CROATIA</i>
Post Office Address	<i>1, allée du Capitaine-Dupont F-92260 Fresnes, France</i>
Inventor's signature	
Date	
Name of fifth inventor	<i>Michael G. TOVEY</i>
Residence	<i>Paris, France</i>
Citizenship	<i>GREAT BRITAIN</i>
Post Office Address	<i>7, rue Lagrange, F-75005 Paris, France</i>
Inventor's signature	
Date	

ANNEX 14

Our registered mail of July 16, 2001 to Mr. BENOIT that was not collected by the latter and returned to us. On the back of the envelope, the acknowledgement of receipt indicates that the mail was not asked for.

PARIS (SIÈGE) :
3, RUE CHAUVEAU-LAGARDE
F - 75008 PARIS
TÉL. : 33 (0)1 44 51 18 00
FAX : 33 (0)1 42 66 08 90
e-mail : info@egyp.fr

LYON (AGENCE) :
62, RUE DE BONNEL
F - 69448 LYON CEDEX 03
TÉL. : 33 (0)4 72 84 97 60
FAX : 33 (0)4 72 84 97 65

ALICANTE (AGENCE) :
PLAZA DEL AYUNTAMIENTO 2-2°-2°
ES - 03002 ALICANTE
TÉL. : 34 965 230 611
FAX : 34 965 230 639

Par RECOMMANDE A/R

Monsieur Patrick BENOIT
24 rue Jonquoy

75014 PARIS

VOTRE REFERENCE :

NOTRE REFERENCE :

LE

B1608ACA – JAZ/VMA/PAD

13 juillet 2001

Objet : Brevet américain n° US 5,919,453 du 6 juillet 1999
Demande divisionnaire n° 09/240,675 déposée le 2 février 1999
au nom de MEDISUP INTERNATIONAL N.V.
Inventeurs : BENOIT, MEYER, MAGUIRE, PLAVEC, TOVEY

BREVETS

Ernest GUTMANN, cpi *
Anne DESAIX, cpi *^o
Carol ALMOND-MARTIN *⁽¹⁾
Julia ANDRAL-ZIURYS ^o
Florence LAZARD, cpi
Jeanne VAILLANT, cpi *^o
Véronique MARCADÉ
Denis BOURGAREL
Carole SELLIN⁽¹⁾

MARQUES, DESSINS

ET MODÈLES

Yves PLASSERAUD, cpi *^o
Martine DEHAUT, cpi ^o
Virginie ZANCAN, cpi ^o
Nathalie PACAUD
Benjamin FONTAINE⁽²⁾
Christophe PELÈSE

DOCUMENTATION

ET VEILLE TECHNOLOGIQUE

Jean-Charles THEODET

* mandataire agréé OEB/EPO

^o US patent attorney

européen en marques

OHM/OHIM

⁽¹⁾ Agence de Lyon

⁽²⁾ Agence d'Alicante

Cher Monsieur,

En tant qu'inventeur désigné dans le brevet américain cité en référence, vous avez signé, le 11 novembre 1994, une déclaration donnant pouvoir à nos correspondants de la société Foley & Lardner, afin qu'ils vous représentent auprès de l'Office Américain des Brevets. Une copie de cette déclaration est ci-jointe.

Dans le cadre d'une demande de brevet divisionnaire, portant sur des peptides particuliers reconnus par l'anticorps monoclonal 64G12, l'Examinateur américain en charge de ce dossier considère que cette déclaration est défectueuse car certaines corrections manuscrites y ont été apportées sans être datées et paraphées. Il nous demande donc de lui fournir une nouvelle déclaration.

Vous trouverez donc ci-joint, une copie du texte initialement déposé ainsi qu'une copie des revendications de la demande divisionnaire. Vous trouverez aussi un exemplaire d'une déclaration supplémentaire, que nous vous prions de bien vouloir nous renvoyer après l'avoir datée et signée. Nous attirons votre attention sur le contenu du sixième paragraphe de cette déclaration, qui précise que vous avez lu et compris la description et l'ensemble des revendications ci-jointes. Si vous avez la moindre question à ce sujet, n'hésitez pas à nous contacter. Par ailleurs, si vous

décidez de ne pas signer cette déclaration, nous vous remercions de bien vouloir nous en avertir.

Veuillez agréer, Cher Monsieur, l'expression de nos meilleures salutations.



Véronique MARCADÉ



Julia ANDRAL-ZIURYS

- P.J. : - Déclaration du 11/11/94
- Texte initialement déposé et revendications de la demande divisionnaire
- Déclaration supplémentaire

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

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WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

(Attorney Docket No. 017283/0123)

the specification of which (check one)

☐ Is attached hereto.

☒ Was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

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THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

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THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

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I HEREBY CLAIM the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

STEPHEN A. BENT	Reg. No. 29,768
DAVID A. BLUMENTHAL	Reg. No. 26,257
BETH A. BURROUS	Reg. No. 35,087
ALAN I. CANTOR	Reg. No. 28,163
WILLIAM T. ELLIS	Reg. No. 26,874
JOHN J. FELDHAUS	Reg. No. 28,822
MICHAEL D. KAMINSKI	Reg. No. 32,904
LYLE K. KIMMS	Reg. No. 34,079
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to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

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I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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Date	

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE 1 INTERFERON

the specification of which (check one)

☐ is attached hereto.

☒ was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
92400902.0	European	31/March/1992	Yes

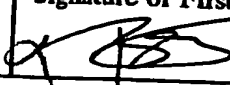
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

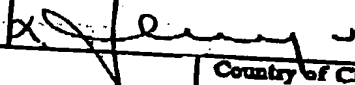
I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; John J. Feldhaus, Reg. No. 28,822; Donald D. Jeffery, Reg. No. 19,980; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Neil M. Roy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 22,665; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115.

Send all correspondence to **FOLEY & LARDNER**, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696. Address telephone communications to Bernhard D. Saxe at (202) 672-5300.

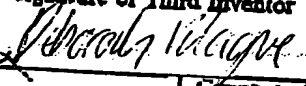
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

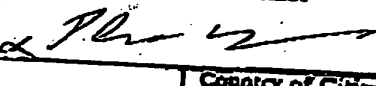
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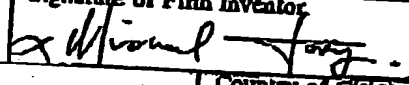
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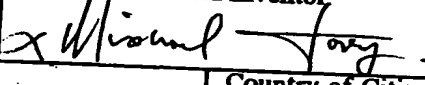
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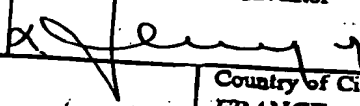
TOVEY


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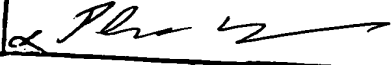
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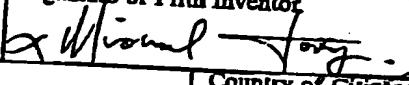
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Docket No. 17283/117/GUPL

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*Revisions de la demande
d'invention*

IN THE CLAIMS:

Please delete claims 1-22 and insert the following new claims:

--23. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 27 to amino acid residue 427 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12 (deposited at the ECACC under no. 92022605).

24. A peptide or polypeptide as claimed in claim 23, consisting of amino acid residue 27 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof.

25. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 1 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12.

26. An analogue of a peptide or polypeptide as claimed in claim 23, which is derived from said peptide or polypeptide by substitution of one or more amino acid residues and which retains the ability to specifically bind to monoclonal antibody 64G12.

27. A method of producing a monoclonal antibody, comprising immunizing an animal with a peptide or polypeptide as claimed in claim 23, fusing spleen cell from the immunized animal with myeloma cells, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide.

28. A method of producing a monoclonal antibody, comprising contacting stimulated B-lymphocytes *in vitro* with a peptide or polypeptide according to claim 23, fusing the resultant B-lymphocytes with B-lymphocytes immortalized with Epstein-Barr

virus, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide. --

IN THE ABSTRACT


Please insert the Abstract provided on the attached sheet.

REMARKS

The Examiner is respectfully requested to enter the above amendments prior to, examination of the instant application. Support for the amendments is present throughout the specification, in particular at pages 10-11.

Respectfully submitted,

February 2, 1999
Date


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ABSTRACT OF THE DISCLOSURE

A monoclonal antibody is provided which is directed against the human interferon type I receptor (IFN-R), which recognizes the extracellular domain of the human IFN-R and which has neutralizing capacity against the biological properties of human type I-IFN. Diagnostic and therapeutic applications for the monoclonal antibody also are provided.

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN: alpha (α), beta (β) and gamma. IFN α and IFN β together are known as the type I interferon.

Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33:251, 1986).

The human IFN α locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, α II or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFN α genes (Weissmann and Weber 1986).

The subtypes of IFN α have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78:2848, 1981) and have the same cellular receptor (Agnat M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFN β) is encoded by a single gene which has approximately 50% homology with the IFN α genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

The interferon gamma (IFN gamma) is also encoded by a single copy, which has little homology with the IFN α and IFN β genes. The receptor for IFN gamma is distinct from the receptor of the α and β interferons.

For the purpose of the present invention the receptor of α and β classes of IFN will be designated IFN-R. This represents natural type I receptor. The group of proteins forming natural interferon α will be designated IFN α , and type I-IFN will represent both natural IFN α , IFN ω , and IFN β .

Despite the fact that interferon is a potent antiviral agent, there is considerable evidence to suggest, that many of the characteristic symptoms of acute virus diseases such as upper respiratory tract infections are caused by an overproduction of interferon alpha. Furthermore, IFN alpha has been shown to contribute to the pathogenesis of certain chronic virus infections in experimental animals and the available evidence suggests that this is also the case for certain human chronic virus diseases such as those due to measles virus.

The interferons α are also potent immunoregulatory molecules which stimulate polyclonal B-cell activation, enhance NK cell cytotoxicity, inhibit T-cell functions, and modulate the expression of the major histocompatibility complex (MHC) class I antigens, all of which are implicated in the induction of autoimmunity and in graft rejection. The abnormal production of interferon α is associated with a number of autoimmune diseases and inflammatory disorders including systemic lupus erythematosus (SLE), type I diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, the acquired immunodeficiency syndrome (AIDS) and severe

combined immunodeficiency disease. The presence of interferon α in the serum of patients with systemic lupus is correlated with both the clinical and humoral signs of increased disease activity. The production of interferon α in HIV positive subjects is also highly predictive of disease evolution.

Administration of interferon α has been reported to exacerbate underlying disease in patients with psoriasis and multiple sclerosis and to induce a SLE like syndrome in patients without a previous history of autoimmune disease. Interferon α has also been shown to induce glomerulonephritis in normal mice and to accelerate the outset of the spontaneous autoimmune disease of NZB/W mice.

Interferon α is also produced during the course of graft-versus-host disease (GVHD) in parallel with the enhanced NK cell activity characteristic of systemic GVHD. Interferon α is the principal modulator of NK cell cytotoxicity and administration of interferon α has been shown to enhance the intestinal consequences of GVHD in normal mice.

The object of the present invention is to provide new antagonists against the biological activities of the human type I-IFN. These antagonists could be used for therapeutical, including prophylaxis purposes, in cases where the type I-IFN (IFN α/β) is abnormally produced and when this abnormal production is associated with pathological symptoms. Such antagonists could also be used for the diagnosis of various diseases or for the study of the evolution of such diseases.

In order to define such antagonists, the inventors have taken into account the fact that the human natural type I-IFN is in fact constituted of a mixture of

interferons (subspecies) and the fact that the composition of this association of different subtypes of interferons varies both quantitatively and qualitatively.

Some natural interferons, such as the ones secreted by Namalwa cells (Namalwa interferon) or leukocyte (leucocyte interferon) have been studied in detail (N.B. Finter and K.H. Fautes, Interferon 2, 1980, p. 65-79 I. Gresser Editor Academic Press ; K. Cantell et al, Interferon 1, 1979 p. 2-25, I. Gresser Editor Academic Press) and were used by the inventors to define natural type I interferons.

In some pathological cases, like AIDS, interferons having some special properties have been described (O.T. Preble et al, Annals of New-York Academy of Sciences p. 65-75). This interferon involved in pathological cases like AIDS nevertheless binds to the same receptor, as described above.

One object of the present invention is to provide an antagonist of the type I-IFN, which would be able to inhibit or neutralize, to a determined extent, the biological properties of the human type I-IFN, that is to say, to neutralize in vivo a mixture of α , β , ω subspecies.

Accordingly the inventors have defined antibodies, especially monoclonal antibodies, which have the property of being antagonists to the type I-IFN. These antibodies are directed against the human type I-IFN receptor.

The invention thus also concerns the use of the monoclonal antibodies for the preparation of pharmaceutical compositions, useful for the treatment of symptoms associated with the abnormal production of

type I-IFN. These monoclonal antibodies are also appropriate for the preparation of diagnosis reagents.

A monoclonal antibody according to the present invention is directed against the human type I-interferon receptor (IFN-R) and is characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

The ability to neutralize the biological properties of type I-IFN can be estimated as a function of the capacity of the monoclonal antibody to neutralize the antiviral activity of the type I-IFN. Such a test is relevant in order to determine whether the antibody assayed is included within the scope of the invention, although it is clear that the biological properties of type I-IFN are not limited to its antiviral properties. Detailed procedures are given in the examples in order to enable to perform such a test of the antiviral activity. The cells tested can advantageously be Daudi-cells, which affinity for the type I-IFN is well known. The main steps of such a test would consist in :

- incubating a determined concentration of human cells responsive to human type I-IFN, with human type I-IFN in the presence of a determined concentration of monoclonal antibodies to be assayed, for a time sufficient to allow the formation of a complex between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the incubated cells with a determined virus, in a determined concentration,
- washing the cells,
- resuspending the cells in culture medium,
- incubating for a time sufficient to allow virus replication ;
- lysing the cells ;
- measuring the virus replication, or measuring the inhibition of the cytopathic effect.

The ability of the monoclonal antibodies of the invention to neutralize the biological properties of the human type I-IFN can be modulated as a function of the dose of antibodies used. Accordingly a 100% inhibition of the biological properties, or a partial inhibition can be obtained.

According to another embodiment of the present invention, the monoclonal antibodies directed against the human type I-IFN receptor, are further characterized by the fact that they are capable of inhibiting the binding of a human type I-IFN, to the human IFN-R.

A monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN to its receptor, can be selected by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies or a hybridoma culture supernatant containing monoclonal antibodies to be assayed, with human cells capable of harboring IFN-R ;
- adding labelled human type I-IFN, in a determined concentration, to the above preincubated medium ;

- incubating the medium containing the human cells, the monoclonal antibodies and the labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the labelled type I-IFN by counting the amount of attached labelled type I-IFN.

Some of the monoclonal antibodies of the invention, have also the capacity to neutralize the antiproliferative properties of the human type I-IFN. This property can also be assayed on Daudi cells, by performing the following steps :

- allowing cells to grow in presence of human type IFN and determined concentration of mAb ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the human type I-IFN.

One property of a monoclonal antibody according to the invention resides in its capacity to recognize the extracellular domain of the human IFN receptor. This property of the monoclonal antibody can be assayed on human cells bearing the natural human receptor but also on the extracellular domain of a recombinant IFN-R such as expressed in a procaryotic cell, for instance in E.coli or a recombinant IFN-R such as expressed in a eucaryotic cell such as mamalian cell for instance a CHO-cell.

This receptor can indeed present different properties, depending on the fact that it is produced in a procaryotic or eucaryotic cell and accordingly

depending on the fact that the post-translational maturation occurred or not. The inventors interestingly showed that relevant assays, to evaluate the capacity of a monoclonal antibody according to the invention i.e. to recognize the cellular IFN-R, can be performed on a recombinant receptor expressed in mamalian cells. As a matter of fact, such recombinant receptor has the same properties as the cellular receptor, as far as its recognizing activity is concerned.

Monoclonal antibodies of the invention can be obtained against various forms of the receptor, including the complete receptor, a particular domain or a peptide characteristic of the aminoacid sequence of the receptor represented in figure 3.

Monoclonal antibodies of the invention can for example be prepared against the soluble form of the receptor. A hydrosoluble polypeptide corresponding to the soluble form of the INF-R is described on figure 2. According to the present invention, a soluble form of the IFN-R corresponds to a peptide or a polypeptide, capable of circulating in the body.

Other monoclonal antibodies according to the invention can also be prepared against a peptide comprised in the extracellular domain of the receptor as described on figure 2. An advantageous peptide corresponds for instance to the aminoacid sequence comprised between aminoacid 1 and aminoacid 229. According to another embodiment of the invention, the antibodies can be prepared against a polypeptide modified by substitution of one or more amino acids, provided that antibodies directed against the non modified extracellular domain of the IFN-R, recognize the modified polypeptide or peptide.

Preferred monoclonal antibodies according to the invention are those which are of the IgG1 type.

Among the antibodies of the invention, an antibody which has the capacity of inhibiting the binding of the type I-IFN to its receptor is preferably characterized in that it inhibits the in vitro binding of human type IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0.5 to 2 $\mu\text{g/ml}$.

The inventors have shown that the high affinity binding capacity of a monoclonal antibody is not sufficient to ensure that this antibody will be able to inhibit the binding activity of the human type I-IFN to the IFN-R. Nevertheless the high affinity binding capacity of the monoclonal antibody is necessary to investigate further the ability of the antibody to inhibit the binding of the type I-IFN to its cellular receptor.

Another monoclonal antibody is characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

According to another embodiment a monoclonal antibody is also characterized in that it neutralizes in vitro the antiproliferative activity of human type IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

A particular group of monoclonal antibodies according to the invention is characterized in that it

neutralizes the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 50 $\mu\text{g/ml}$, preferably 1 to 20 $\mu\text{g/ml}$, for a concentration of type I-IFN in the range of 1 to 1000 units with reference to the international standard MRC 69/19.

Advantageously, the monoclonal antibody according to the invention is such that these antibodies do not bind to the human receptor for IFN gamma.

One particular antibody satisfying the requirements of the invention, is such as it directed against an epitope on the amino-acid sequence comprised between amino-acid 27 and amino-acid 427 of the extracellular domain of the human IFN-R as represented on figure 2.

One particularly interesting monoclonal antibody according to the invention is the antibody designated 64G12 under n° 92022605 which has been deposited at the ECACC (European Collection of Animal Cell Cultures Porton Down Salisbury, Wiltshire SP4 056, United Kingdom) on February 26, 1992.

These antibodies may be prepared by conventional methods involving the preparation of hybridoma cells by the fusion of myeloma cells and spleen cells of an animal immunized beforehand with the peptide antigen, on the conditions such that the antigen against which the antibodies are formed is constituted by the extracellular domain of IFN-R or any polypeptide or peptide of this domain.

The hybridomas are constructed according to the protocole of Kohler and Milstein (Nature, 1974, 256: 495-497). For example the hybridomas are derived from

the fusion of the spleen cells above described with NS1 mouse (BalbC) HGPRT⁻ as myeloma cell.

A second procedure for the production of monoclonal antibodies according to the invention, consists in carrying out the fusion between B-cells of blood immortalized with the Epstein/Barr virus and human B lymphocytes placed beforehand in contact with the extracellular domain or a fragment thereof of the IFN-R, against which it is decided to form monoclonal antibodies. B-cells placed in contact beforehand with the extracellular domain of IFN-R or fragment thereof against which it is decided to form monoclonal antibodies, may be obtained by in vitro culture contacted with the antigens, the recovery of the B-cells coated with these antigens being preceded by one or several cycles of stimulation.

The invention thus concerns human antibodies as obtained by carrying out the above procedure, having the above defined properties.

The invention also aims at providing a monoclonal antibody characterized in that the variable or complementary determining regions of its heavy and/or light chains are grafted on the framework and/or constant regions of a human antibody.

The invention further provides a composition having antagonist properties for the biological properties of the human type I-IFN, characterized in that it comprises monoclonal antibodies as defined above.

Accordingly the invention provides a pharmaceutical composition characterized in that it comprises monoclonal antibodies as defined above, together with an appropriate pharmaceutical vehicle.

The invention also concerns the use of a monoclonal antibody as defined above, for the manufacture of a drug for the treatment or profilaxis of a pathological state or symptoms associated with overproduction of type-I-IFN.

According to a first example, the antibodies can be used in a pharmaceutical composition, for the treatment of allograft rejection.

According to another example, antibodies of the invention are used as active principle in a pharmaceutical composition for the treatment of autoimmune and inflammatory diseases. Such diseases include systemic lupus erythematosus, type 1 diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, acquired immunodeficiency syndrome (AIDS), and severe combined immunodeficiency disease.

Treatment of acute virus diseases can also be performed with the antibodies of the invention. As example upper respiratory tract infections, chronic virus infections such as those due to measles virus, can be performed.

The antibodies of the invention can also be used for the in vitro diagnosis of the presence of the human type I-IFN receptor or cells.

Further details and additional information will arise from the description from the description of the examples and from the figures.

FIGURES

- Figure 1 : binding of ^{125}I -labelled monoclonal antibodies 34F10 and 64G12 to :

- A : Daudi cells
- B : Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was measured by incubation with a 100 fold excess of cold antibodies and subtracted from total counts.

- Figure 2 : nucleotide and corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthesized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in figure 2.

- Figure 3 : nucleotide and corresponding amino-acid sequence of the human IFN-R.

E X A M P L E S**EXAMPLE 1 :**Synthesis of the soluble receptorsSynthesis in E.coli

A fragment of DNA containing the sequence coding for the extracellular domain (amino acids 27 to 427) of the human INF-R (figure 2), in which an extra-sequence coding for 5 histidyl residues was introduced just before the termination codon, was cloned in the expression vectors pKK233-2. This fragment was produced by the Polymerase Chain Reaction (PCR) and the resulting plasmids were sequenced to confirm both in-frame insertion with the Shine-Dalgarno sequence and the appropriate sequence coding for the receptor.

The poly-histidyl tail introduced into the recombinant protein enables it to be purified rapidly by affinity chromatography on a chelated nickel support (NTA column) as described previously (Hochuli E. et al, Bio/technology, 1988, 1321-1325).

The plasmid was introduced into the E.coli strain, JM105, and protein synthesis induced by addition of IPTG to the culture medium (pKK233-2, tac promoter).

Proteins were extracted from the bacterial pellet and the soluble receptor purified to homogeneity by affinity chromatography as described hereafter. This procedure yielded a protein that migrates as 2 bands around 50 kDa under reducing conditions and three bands under non-reducing conditions. The maximum concentration of the protein obtained by different procedures was approximately 20µg/ml.

The N-terminal sequence of the two proteins detected by gel electrophoresis has shown that both proteins are the expected fragment of the receptor.

Synthesis and purification of an unglycosylated soluble receptor :

Bacterial culture (250ml)

|

IPTG induction 3h

|

cell pellet

6M Guanidine hydrochloride pH8

|

centrifugation

|

NTA column:

Washes pH 8 urea 8M

pH 6,3 urea 8M

pH 5.9 urea 8M

|

Elution pH 4 urea 8M

|

refolding dilution, dialysis

|

against Tris 0,1 M pH9

dialysis PBS

Using the same PCR approach, we also constructed an expression vector coding for the IFN-R amino acid sequence 1-427, with an additional 5-histidyl residues at the C-terminus, inserted in expression vector pXMT-3. The exact nucleotide sequence of the insert was also confirmed.

The resulting plasmid was introduced by electroporation into Cos7 cells for transient expression and the recombinant protein was purified to homogeneity by affinity chromatography followed by ion exchange chromatography on mono-Q (Pharmacia) as described hereafter.

Purification of the soluble IFN-R from Cos7 cells

preparative electroporation of
cos cells

18 h

serum free medium

supernatants taken after 48h, 72h, 96h

concentration

NTA column

Wash PBS

elution 0.1 M NaOAc pH 5.5

neutralization

concentration, 30 000 cut off

Mono Q (0-0.5 M Na Cl)

This purification yielded to a 76 kDa protein whose N-terminal sequence corresponds to the predicted receptor sequence with some heterogeneity in the processing of the leader sequence.

EXAMPLE 2 :

Production of monoclonal antibodies against the interferon type I receptor

1) Production of the monoclonal antibodies

Mice were immunized by injection of recombinant soluble interferon (r sIFN-R) purified from E.coli or from a culture supernatant of Cos7 cells. Initially mice were injected both intraperitoneally and subcutaneously with the purified protein in complete Freund's adjuvant. Subsequently mice were injected once a week intraperitoneally with the purified proteins diluted in buffered saline solution. Ten micrograms of recombinant proteins were injected each time.

After the fourth injection, blood was collected and the presence of specific serum antibodies were tested by both ELISA and Western blot against the recombinant receptor. The strongest responders were then boosted with a total of 10 μ g of antigen half of which was injected intravenously and half intraperitoneally.

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT⁻ myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35:1-32, 1980). Briefly, 5×10^7 spleen cells were fused to 3×10^7 myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as 20×10^7 spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method :

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.05% Tween 20,

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% Tween 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

3) Identification of reactivity to the natural interferon type I receptor

The reactivity of the monoclonal antibodies (mAbs) recognizing the recombinant sIFN-R was tested against the natural class I receptor expressed at the surface of Daudi cells, by membrane immunofluorescence. Briefly, 5×10^5 Daudi cells were incubated in 100 μ l of culture supernatant of chosen hybridomas for 30 min at 4°C. The cells were then washed 4 times in RPMI medium containing 1% BSA and further incubated with a diluted FITC labelled goat anti-mouse F(ab')₂ for 30 min at 4°C. The cells were finally analyzed by flow cytometry after washing. One of the 35 tested antibodies produced against the E.coli recombinant receptor and 5 of the 6 tested antibodies produced against the COS recombinant receptor were found to recognize the natural receptor on the Daudi cells.

Cloning of these hybridomas was then performed by limiting dilution. The isotype of these mAbs was determined by an ELISA method using isotype specific antibodies. All 6 mAbs were found to be IgG1 with kappa light chains. A summary of the reactivity of these 6 mAbs is given in Table 1.

Monoclonal antibodies were purified from culture supernatants by protein G chromatography.

Table 1 :Reactivity of the anti IFN-R monoclonal antibodies

	Reactivity against the recombinant receptor				Reactivity against * the cellular receptor
	E.COLI		COS		
	ELISA	Western	ELISA	Western	immunofluorescence
34F10	+	+	+	+	+
64G12	+	+	+	+	+
63F6 64G2 64D10 65D8	-	-	+	+	+
				weak	

* measured on Daudi cells

EXAMPLE 3 :Inhibition of the binding of interferon to human cell lines

Inhibition of interferon binding to human cells was assayed as follows. 10^6 cells were preincubated at 4°C for 30 min with various dilutions of hybridoma culture supernatants or purified mAbs or with medium alone. ^{125}I -labelled IFN alpha 8 or alpha 2 was added at the concentration of 100pM and cells incubated for a further 2 hours at 4°C. These incubations were performed in RPMI medium containing 20mM HEPES pH 7.4 and 10% foetal calf serum (FCS). The cells were finally washed 4 times with RPMI - 1% FCS and counted to determine bound radioactivity.

The mAb secreted by the hybridoma line 64G12 (latter named mAb 64G12) was shown in this assay to inhibit the binding of labelled IFN to the cells in a dose-dependent manner. 50% inhibition of binding to the Daudi cells (Burkitt lymphoma cell line ; Klein et al., Cancer Research, 28:1300-1310, 1968) was obtained at a mAb concentration of 0.4 μ g/ml. The same inhibition was obtained with K562 cells (chronic myelogenous leukemia, Lozzio and Lozzio, Cell, 45:321-334, 1975) but 50% inhibition was obtained at 11 μ g/ml for HL60 cells (Promyelocytic leukemia, Collins S.J. et al., Nature, 270:347-349, 1977) and 60 μ g/ml for Ly28 cells (Klein G. et al. Int. J. Cancer, 10:44-57, 1972).

Table 2 :

The inhibition of binding of labelled IFN alpha 2 to various cell lines by mAB64G12

Cell lines	Concentration of mAB which gives 50% inhibition of binding
Daudi K562	0,4 μ g/ml
HL60	11 μ g/ml
Ly28	60 μ g/ml

The difference in the mAb concentration at which 50% inhibition of binding of IFN is obtained has been investigated by direct binding of ¹²⁵I-labelled mABs 64G12 and 34F10 to the same cell lines and Scatchard

plot analysis of the results. In the concentration range of 0.1 to 1.5 $\mu\text{g/ml}$, a high affinity binding of the mAb 34F10 ($\approx 10\text{nM}$) was seen on all cell lines whereas a high affinity binding of mAb 64G12 was only detected on Daudi and K562 cells (Figure 1).

EXAMPLE 4 :

Inhibition of the function of type I interferon

Functional inhibition of type I interferon by the purified mAb 64G12 was demonstrated in an antiviral assay on Daudi cells using either recombinant IFN alpha 2, IFN beta and IFN omega, or purified Namalwa and leucocyte interferons, and in an antiproliferative assay with recombinant IFN alpha 2.

* Antiviral activity

An antiviral assay on Daudi cells was performed as described (M. Dron and M.G. Tovey, J. Gen. Virol. 64:2641-2647, 1983). Cells ($0.5 \times 10^6/\text{ml}$) were incubated for 24 hours in the presence of interferon and antibodies. 10^6 cells in 1 ml were then infected for 1 hour at 37°C with Vesicular stomatitis virus (VSV) then washed 3 times, resuspended in culture medium and incubated for 18 hours at 37°C . Cells were then lysed by freeze-thawing and virus replication measured by titration of the supernatants on L929 cells. A dose-dependent inhibition of the antiviral activity of the various subtypes of type I IFN was demonstrated for the purified mAb 64G12.

For the antiviral assay with the Wish cells, cells were incubated for 24 hours with various concentrations of interferons in the presence of the mAbs prior to challenge with VSV. In this assay, the mAb 64G12 was demonstrated to block completely the antiviral activity of Leukocyte IFN (50U/ml), recombinant IFN alpha 2 (50U/ml) and interferon from the sera of AIDS patients (50, 75 and 150U/ml).

* antiproliferative activity

For the antiproliferative assay, Daudi cells were seeded at a concentration of 10^5 cells per ml in a 96 well plate in the presence of interferon and purified inhibitory or control antibody. Cells were then counted after 24, 48 and 72 hours with a Coulter counter and checked for viability by trypan blue exclusion. Purified mAb 64G12 demonstrated a dose-dependent inhibition of the antiproliferative activity of interferon alpha 2.

C L A I M S

1. Monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

2. Monoclonal antibody directed against the human type I IFN-R according to claim 1, characterized by its capacity to inhibit the binding of a human pathological type I-IFN, to the IFN-R.

3. Monoclonal antibody according to claim 1 or 2, which is obtainable from a hybridoma cell prepared by fusion of a myeloma cell with spleen cells from an animal previously immunized with the soluble form of the human IFN-R.

4. Monoclonal antibody according to anyone of claims 1, 2 or 3, characterized in that it recognizes an epitope on a soluble form of the human cellular IFN-R or of a recombinant IFN-R.

5. Monoclonal antibody according to anyone of claims 1 to 4, characterized in that it inhibits in vitro the binding of human type I-IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0,5 to 2 $\mu\text{g/ml}$.

6. Monoclonal antibody according to anyone of claims 1 to 5, characterized in that it neutralizes in vitro the antiproliferative activity of the human type I-IFN, on cells highly responsive to this human type I-IFN,

for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

7. Monoclonal antibody according to anyone of claims 1 to 6, characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells poorly responsive to this human type I-IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

8. Monoclonal antibody according to anyone of claims 1 to 7, characterized in that it does not bind to the human receptor of the IFN gamma.

9. Monoclonal antibody according to anyone of claims 1 to 8, characterized in that it recognizes an epitope on the aminoacid sequence 27 to 427 of the human IFN-R.

10. Monoclonal antibody according to anyone of claims 1 to 9, characterized in that it neutralizes in vitro the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

11. Monoclonal antibody according to anyone of claims 1 to 10, characterized in that it neutralizes in vitro the antiviral activity of the human class I-IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

12. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is the 64G12 antibody, deposited at the ECACC on February 26, 1992 under n° 92022605.

13. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a humanized antibody, for instance characterized in that the variable or complementary determining regions of its

heavy and light chains are grafted on the framework and constant regions of a human antibody.

14. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a human antibody.

15. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is an IgG1 type antibody.

16. Hybridoma cell, characterized in that it produces monoclonal antibodies according to claims 1 to 13.

17. Composition having antagonist properties to the type I-IFN, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 16.

18. Pharmaceutical composition, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 17, together with an appropriate pharmaceutical vehicle.

19. Use of a monoclonal antibody according to anyone of claims 1 to 17, for the manufacture of a drug for the treatment or prophylaxis of a pathological state associated with proliferative cell activity and/or viral cell infection.

20. Process for the selection of a monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN, to the IFN-R, characterized by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies according to anyone of claims 1 to 15 or a hybridoma culture supernatant containing monoclonal antibodies, with human cells susceptible of harboring IFN-R ;
- adding labelled human type I-IFN in a determined concentration, to the above preincubating medium ;

- incubating the medium containing the human cells, monoclonal antibodies and labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the type I-IFN, by counting the amount of attached labelled type I-IFN.

21. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiproliferative activities of the type I-IFN, on human cells characterized by the steps of :

- allowing cells to grow in the presence of human type I-IFN and in the presence of a determined concentration of monoclonal antibodies according to anyone of claims 1 to 15 ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the type I-IFN.

22. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiviral activities of the natural, non pathological or pathological type I-IFN on human cells, characterized by the steps of :

- incubating cells with type I-IFN and monoclonal antibodies according to anyone of claims 1 to 15, in determined concentrations, for a time sufficient to allow the formation of a complex

between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the above incubated cells with a determined concentration of a virus ;
- washing the cells ;
- resuspending the cells in culture medium ;
- incubating for a time sufficient to allow the replication of the virus ;
- lysing the cells and ;
- measuring the virus replication or measuring the inhibition of the cytopathic effect.

A B R I D G M E N TMONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The invention relates to a monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

It further concerns their use for the diagnosis.

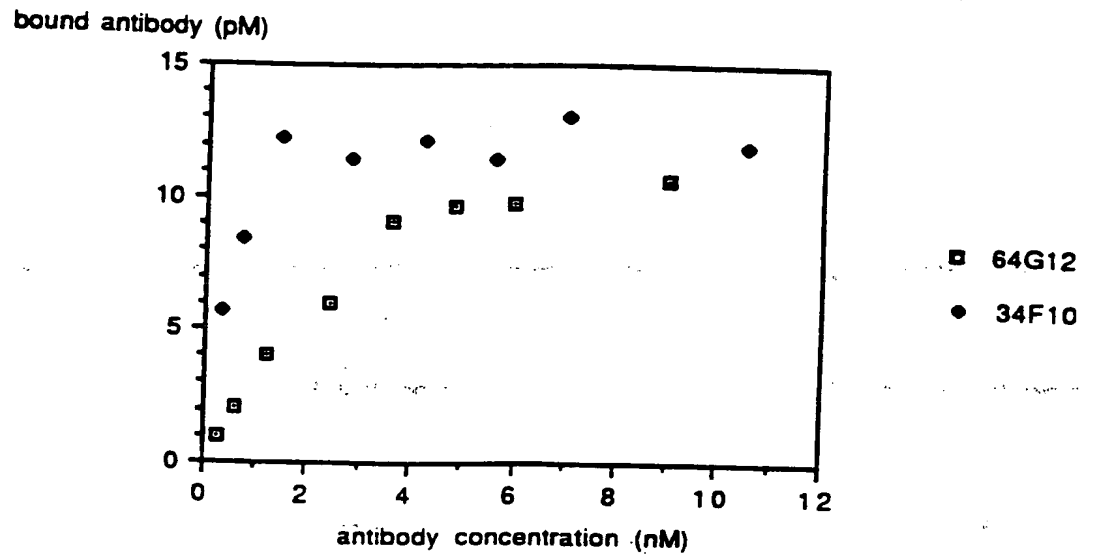
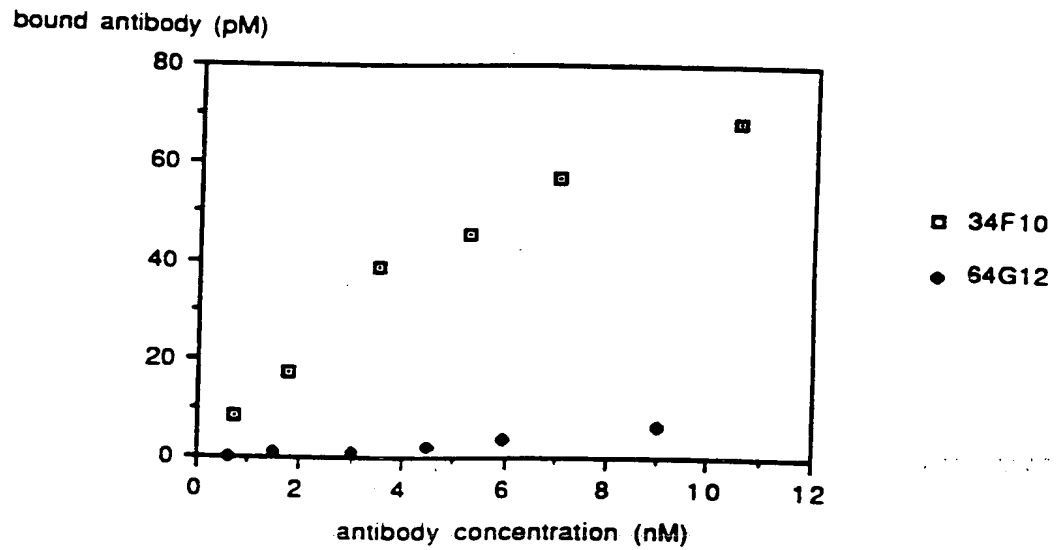
A**B**

FIGURE 1

CTGCAGGCATCTGCGCGGCTCCAC

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 2A

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn MET Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu

AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr MET Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA TGAGGTACC
Asn Thr Ser Lys

1324

FIGURE 2B

CTGCAGGGATCTCGGCGGCTCCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

ATT GAA AAT ATA AGC ACA ATT GCT ACA GTA GAA GAA ACT AAT CAA ACT GAT GAA
 Ile Glu Asn Ile Ser Thr Ile Ala Thr Val Glu Glu Thr Asn Gln Thr Asp Glu

GAT CAT AAA AAA TAC AGT TCC CAA ACT AGC CAA GAT TCA GGA AAT TAT TCT AAT
 Asp His Lys Lys Tyr Ser Ser Gln Thr Ser Gln Asp Ser Gly Asn Tyr Ser Asn

GAA GAT GAA AGC GAA AGT AAA ACA AGT GAA GAA CTA CAG CAG GAC TTT GTA TCA
 Glu Asp Glu Ser Glu Ser Lys Thr Ser Glu Glu Leu Gln Gln Asp Phe Val

CCAGAAATGAACTGTGTCAAGTATAAGGTTTTTCAGCAGGAGTTACACTGGTACC

1697

FIGURE 3C

LA POSTE

AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDÉ

RA 6156 2080 1FR

Présenté le :

10/01/01

Distribué le :

Signature du destinataire :

RETOUR A :

MANN - 1400

Rue Chateaubriand - 14000

14000 PARIS

SIREN 356 000 000 RCS NANTERRE

AVIS DE RÉCEPTION

AR




26

ANNEX 15

Translation from French into English of the back of the envelope mentioned in Annex 14.

Annex 15: Non-collected mail

Acknowledgement of Receipt
of your Registered Mail

LA POSTE 

RA 6156 2080 1FR
DZAKHNA BLOUACH

**AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDE**

Présenté le : 12/07/01

Distribué le : _____

Signature du destinataire : _____

RETOUR A
M. L. HANN - P. ASSERIAUD
5 rue Chateaubriand - 75002 PARIS

RETOUR A
M. L. HANN - P. ASSERIAUD
5 rue Chateaubriand - 75002 PARIS

RETOUR A
M. L. HANN - P. ASSERIAUD
5 rue Chateaubriand - 75002 PARIS

RETOUR A
M. L. HANN - P. ASSERIAUD
5 rue Chateaubriand - 75002 PARIS

Return to Sender

Not asked for
Return to Sender



Stamp showing that the mail
was still at the Post Office
on August 2, 2001.

VERIFICATION OF TRANSLATION

I, Julia ANDRAL-ZIURYS, working at ERNEST GUTMANN-YVES PLASSERAUD S.A., 3 rue Chauveau-Lagarde, 75008 Paris (France),

declare that I am conversant with the French and English languages and that to the best of my knowledge and belief the following (Annex 15) is a true translation of the indications on the back of the envelope of our registered mail of July 16, 2001 to Mr. BENOIT that was not collected by the latter and returned to us (Annex 14).

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application or any patent issuing thereon.

Paris, November 22, 2001

Signature:


A handwritten signature in black ink, appearing to read "Julia Andral-Ziurys", written in a cursive style.

Julia ANDRAL-ZIURYS 22/11/01

ANNEX 16


Acknowledgement of receipt n° 6156 2267 4FR of our registered mail of September 4, 2001, to Mr. BENOIT, and collected on September 8, 2001.

Ann x 16 : Acknowledgement of receipt n° 6156 2267 4FR of our registered mail of September 4, 2001, to Mr. BENOIT, and collected on September 8, 2001.

LA POSTE 

RA 6156 2267 4FR
B1603ACA / JMS + MH/MNH

**AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDÉ**

Présenté le : 6.09.01
Distribué le : 08.09.2001
Signature du destinataire : 

RETOUR A
M. Patrick Benoit
24 rue Senguer
75014 Paris
Gutmann - Plussard
3 rue Chateaubriand
75008 Paris


PARIS ALESIA
1000
17
1745

SIREN 3861000 000 RCS NANTERRE

AVIS DE RÉCEPTION

ANNEX 17

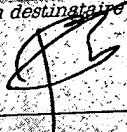
Acknowledgement of receipt n° 6156 2427 4FR of our registered mail of October 5, 2001, to Mr. BENOIT, and collected on October 13, 2001.

LA POSTE 

AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDE

PARIS ALÉS
12
13-10
2001
RUE D'ALF

RA 6156 2427 8FR
Jus/VNA/B1608ACA

Présenté le : 9.10.01
Distribué le : 13/10/01
Signature du destinataire: 

~~M. Patrick Benoit
24 Rue Souffroy
75004 Paris~~

RETOUR A
gutmann-Plasseand
3 Rue Chauveau Lagarde
75008 Paris

SIREN 696 000 000 RCS NANTERRE

AR
AVIS DE RÉCEPTION

ANNEX 18

Our registered mail of July 16, 2001 to Ms. MAGUIRE, which was not collected by the latter and was returned to us on August 6, 2001. On the back of the envelope, the acknowledgement of receipt indicates that the mail was not asked for.



ERNEST GUTMANN - YVES PLASSERAUD S.A.
SOCIETE DE CONSEILS EN PROPRIETE INDUSTRIELLE

PARIS (SIÈGE) :
3, RUE CHAUVEAU-LAGARDE
F - 75008 PARIS
TÉL. : 33 (0)1 44 51 18 00
FAX : 33 (0)1 42 66 08 90
e-mail : info@egyp.fr

LYON (AGENCE) :
62, RUE DE BONNEL
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FAX : 33 (0)4 72 84 97 65

BDS

ALICANTE (AGENCE) :
PLAZA DEL AYUNTAMIENTO 2-2°-2°
ES - 03002 ALICANTE
TÉL. : 34 965 230 611
FAX : 34 965 230 639

Par RECOMMANDÉ A/R

Madame Deborah MAGUIRE
24 rue Maître-Albert

75005 PARIS

VOTRE REFERENCE :

NOTRE REFERENCE :

LE

B1608ACA – JAZ/VMA/PAD

13 juillet 2001

Objet : Brevet américain n° US 5,919,453 du 6 juillet 1999
Demande divisionnaire n° 09/240,675 déposée le 2 février 1999
au nom de MEDISUP INTERNATIONAL N.V.
Inventeurs : BENOIT, MEYER, MAGUIRE, PLAVEC, TOVEY

BREVETS

Ernest GUTMANN, cpi *
Anne DESAIX, cpi *
Carol ALMOND-MARTIN *
Julia ANDRAL-ZIURYS *
Florence LAZARD, cpi
Jeanne VAILLANT, cpi *
Véronique MARCADÉ
Denis BOURGAREL
Carole SELLIN *

MARQUES, DESSINS
ET MODÈLES

Yves PLASSERAUD, cpi *
Martine DEHAUT, cpi *
Virginie ZANCAN, cpi *
Nathalie PACAUD
Benjamin FONTAINE
Christophe PELÈSE

DOCUMENTATION
ET VEILLE TECHNOLOGIQUE

Jean-Charles THEODET

Chère Madame,

En tant qu'inventeur désigné dans le brevet américain cité en référence, vous avez signé, le 11 novembre 1994, une déclaration donnant pouvoir à nos correspondants de la société Foley & Lardner, afin qu'ils vous représentent auprès de l'Office Américain des Brevets. Une copie de cette déclaration est ci-jointe.

Dans le cadre d'une demande de brevet divisionnaire, portant sur des peptides particuliers reconnus par l'anticorps monoclonal 64G12, l'Examinateur américain en charge de ce dossier considère que cette déclaration est défectueuse car certaines corrections manuscrites y ont été apportées sans être datées et paraphées. Il nous demande donc de lui fournir une nouvelle déclaration.

Vous trouverez donc ci-joint, une copie du texte initialement déposé ainsi qu'une copie des revendications de la demande divisionnaire. Vous trouverez aussi un exemplaire d'une déclaration supplémentaire, que nous vous prions de bien vouloir nous renvoyer après l'avoir datée et signée. Nous attirons votre attention sur le contenu du sixième paragraphe de cette déclaration, qui précise que vous avez lu et compris la description et l'ensemble des revendications ci-jointes. Si vous avez la moindre question à ce sujet, n'hésitez pas à nous contacter. Par ailleurs, si vous

mandataire agréé OEB/EPO
US patent attorney
conseil européen en marques
OHM/OHIM

* Agence de Lyon
* Agence d'Alicante

SOCIETE ANONYME
AU CAPITAL DE 3 000 000 F
RCS PARIS B 332 417 500
APE 741 A

Acquisition et défense des droits, stratégie de protection, liberté d'exploitation
et recherches de disponibilité, oppositions, consultations, contrats et audits

décidez de ne pas signer cette déclaration, nous vous remercions de bien vouloir nous en avvertir.

Veuillez agréer, Chère Madame, l'expression de nos meilleures salutations.



Véronique MARCADÉ



Julia ANDRAL-ZIURYS

- P.J. : - Déclaration du 11/11/94
- Texte initialement déposé et revendications de la demande divisionnaire
- Déclaration supplémentaire

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR,
WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

(Attorney Docket No. 017283/0123)

the specification of which (check one)

☐ Is attached hereto.

☒ Was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code §119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
92400902.0	European	31/March/1992	Yes	NO

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

STEPHEN A. BENT	Reg. No. 29,768
DAVID A. BLUMENTHAL	Reg. No. 26,257
BETH A. BURROUS	Reg. No. 35,087
ALAN I. CANTOR	Reg. No. 28,163
WILLIAM T. ELLIS	Reg. No. 26,874
JOHN J. FELDHAUS	Reg. No. 28,822
MICHAEL D. KAMINSKI	Reg. No. 32,904
LYLE K. KIMMS	Reg. No. 34,079
KENNETH E. KROSIN	Reg. No. 25,735

JOHNNY A. KUMAR	Reg. No. 34,649
JACK LAHR	Reg. No. 19,621
GLENN LAW	Reg. No. 34,371
PETER G. MACK	Reg. No. 26,001
STEPHEN B. MAEBIUS	Reg. No. 35,264
BRIAN J. MC NAMARA	Reg. No. 32,789
SYBIL MELOY	Reg. No. 22,749
RICHARD C. PEET	Reg. No. 35,792
GEORGE E. QUILLIN	Reg. No. 32,792
ANDREW E. RAWLINS	Reg. No. 34,702
BERNHARD D. SAXE	Reg. No. 28,665
CHARLES F. SCHILL	Reg. No. 27,590
RICHARD L. SCHWAAB	Reg. No. 25,479
MICHELE M. SIMKIN	Reg. No. 34,717
HAROLD C. WEGNER	Reg. No. 25,258

to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

I request that all correspondence be directed to:

Bernhard D. Saxe
Foley & Lardner
At Washington Harbour
3000 K Street, NW, Suite 500
Washington, DC 2000

Telephone: 202-672-5472
Facsimile: 202-672-5399

I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Name of first inventor	<u>Patrick BENOIT</u>
Residence	<u>Paris, France</u>
Citizenship	<u>FRANCE</u>
Post Office Address	<u>24, rue Jonquoy, F-75014 Paris, France</u>
Inventor's signature	<u></u>
Date	<u></u>

Name of second inventor	<i>Francois MEYER</i>
Residence	<i>Paris, France</i>
Citizenship	<i>LUXEMBOURG</i>
Post Office Address	<i>3 Place du Panthéon, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of third inventor	<i>Deborah MAGUIRE</i>
Residence	<i>Paris, France</i>
Citizenship	<i>AUSTRALIA</i>
Post Office Address	<i>24, rue Maitre-Albert, F-75005 Paris, France</i>
Inventor's signature	<i>X</i>
Date	<i>X</i>
Name of fourth inventor	<i>Ivan PLAVEC</i>
Residence	<i>Fresnes, France</i>
Citizenship	<i>CROATIA</i>
Post Office Address	<i>1, allée du Capitaine-Dupont F-92260 Fresnes, France</i>
Inventor's signature	
Date	
Name of fifth inventor	<i>Michael G. TOVEY</i>
Residence	<i>Paris, France</i>
Citizenship	<i>GREAT BRITAIN</i>
Post Office Address	<i>7, rue Lagrange, F-75005 Paris, France</i>
Inventor's signature	
Date	

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE 1 INTERFERON

the specification of which (check one)

☐ is attached hereto

☒ was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
92400902.0	European	31/March/1992	Yes

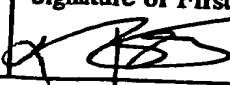
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; John J. Feldhaus, Reg. No. 28,822; Donald D. Jeffery, Reg. No. 19,980; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Hil Meloy, Reg. No. 22,749; George E. Quinn, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 22,115; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115.

Send all correspondence to **FOLEY & LARDNER**, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696.
Address telephone communications to Bernhard D. Saxe at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor Patrick BENOIT		Signature of First or Sole Inventor 	Date 11/11/94
Residence Address Paris, France		Country of Citizenship FRANCE	
Post Office Address 24, rue Jonquoy, F-75014 Paris, France			

Signatures should conform to names as typewritten. ☒ Additional inventors on attached Page 2.


Full Name of Second Inventor Francois MEYER	Signature of Second Inventor <i>[Signature]</i>	Date <i>[Date]</i>
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 14, square Adanson, F-75005 Paris, France		


Deborah


Full Name of Third Inventor Deborah-MAGUIRE	Signature of Third Inventor <i>[Signature]</i>	Date <i>11/16/94</i>
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 24, rue Maitre-Albert, F-75005 Paris, France		

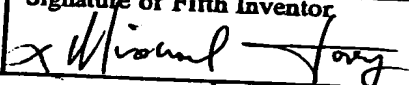
Full Name of Fourth Inventor Ivan PLAVEC	Signature of Fourth Inventor <i>[Signature]</i>	Date <i>11/11/94</i>
Residence Address Fresnes, France	Country of Citizenship FRANCE CROATIA	
Post Office Address 1, allée du Capitaine-Dupont F-92260 Fresnes, France		

Full Name of Fifth Inventor Michael G. TOVEY	Signature of Fifth Inventor <i>[Signature]</i>	Date <i>[Date]</i>
Residence Address Paris, France	Country of Citizenship GREAT BRITAIN	
Post Office Address 6, rue des Quatrefages, F-75005 Paris, France		

Full Name of Second Inventor Francois MEYER	Signature of Second Inventor 	Date
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 14, square Adanson, F-75005 Paris, France		

Full Name of Third Inventor Deborah MAGUIRE	Signature of Third Inventor 	Date
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 24, rue Maitre-Albert, F-75005 Paris, France		

Full Name of Fourth Inventor Ivan PLAVEC	Signature of Fourth Inventor 	Date
Residence Address Fresnes, France	Country of Citizenship FRANCE	
Post Office Address 1, allée du Capitaine-Dupont F-92260 Fresnes, France		

Full Name of Fifth Inventor Michael G. TOVEY	Signature of Fifth Inventor 	Date 11/1/94
Residence Address Paris, France	Country of Citizenship GREAT BRITAIN	
Post Office Address 5, rue des Quatrefages, F-75005 Paris, France		

08/11 '94 13:17

47287602

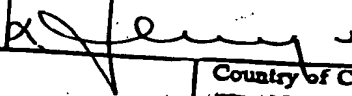
TOVEY


0001/001

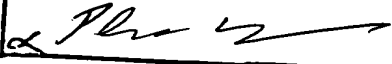
PAGE 2

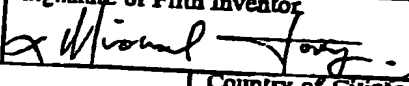
B 1608 AC

Docket No. 17283/117/GUPL

Full Name of Second Inventor Francois MEYER	Signature of Second Inventor 	Date 11/11/94
Residence Address Paris, France	Country of Citizenship FRANCE LYNETBOURG	
Post Office Address 14, square Adanson, F-75005 Paris, France		

Full Name of Third Inventor Dehborah MAGUIRE	Signature of Third Inventor 	Date 11/11/94
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 24, rue Maitre-Albert, F-75005 Paris, France		

Full Name of Fourth Inventor Ivan PLAVEC	Signature of Fourth Inventor 	Date 11/11/94
Residence Address Fresnes, France	Country of Citizenship FRANCE CROATIA	
Post Office Address 1, allée du Capitaine-Dupont F-92260 Fresnes, France		

Full Name of Fifth Inventor Michael G. TOVEY	Signature of Fifth Inventor 	Date 11/11/94
Residence Address Paris, France	Country of Citizenship GREAT BRITAIN	
Post Office Address 6, rue des Quatrefages, F-75005 Paris, France		

*Revendications de la demande
déclaratoire*

IN THE CLAIMS:

Please delete claims 1-22 and insert the following new claims:

--23. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 27 to amino acid residue 427 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12 (deposited at the ECACC under no. 92022605).

24. A peptide or polypeptide as claimed in claim 23, consisting of amino acid residue 27 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof.

25. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 1 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12.

26. An analogue of a peptide or polypeptide as claimed in claim 23, which is derived from said peptide or polypeptide by substitution of one or more amino acid residues and which retains the ability to specifically bind to monoclonal antibody 64G12.

27. A method of producing a monoclonal antibody, comprising immunizing an animal with a peptide or polypeptide as claimed in claim 23, fusing spleen cell from the immunized animal with myeloma cells, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide.

28. A method of producing a monoclonal antibody, comprising contacting stimulated B-lymphocytes *in vitro* with a peptide or polypeptide according to claim 23, fusing the resultant B-lymphocytes with B-lymphocytes immortalized with Epstein-Barr

virus, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide. --

IN THE ABSTRACT

Please insert the Abstract provided on the attached sheet.

REMARKS

The Examiner is respectfully requested to enter the above amendments prior to examination of the instant application. Support for the amendments is present throughout the specification, in particular at pages 10-11.

Respectfully submitted,

February 2, 1999

Date



Bernhard D. Saxe
Reg. No. 28,665

FOLEY & LARDNER
3000 K Street, N.W.
Suite 500
Washington, D.C. 20007-5109
Tel: (202) 672-5300

ABSTRACT OF THE DISCLOSURE

A monoclonal antibody is provided which is directed against the human interferon type I receptor (IFN-R); which recognizes the extracellular domain of the human IFN-R and which has neutralizing capacity against the biological properties of human type I-IFN. Diagnostic and therapeutic applications for the monoclonal antibody also are provided.

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN : alpha (α), beta (β) and gamma. IFN α and IFN β together are known as the type I interferon.

Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes, with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33:251, 1986).

The human IFN α locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, α II or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFN α genes (Weissmann and Weber 1986).

The subtypes of IFN α have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78:2848, 1981) and have the same cellular receptor (Agnet M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFN β) is encoded by a single gene which has approximately 50% homology with the IFN α genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

The interferon gamma (IFN gamma) is also encoded by a single copy, which has little homology with the IFN α and IFN β genes. The receptor for IFN gamma is distinct from the receptor of the α and β interferons.

For the purpose of the present invention the receptor of α and β classes of IFN will be designated IFN-R. This represents natural type I receptor. The group of proteins forming natural interferon α will be designated IFN α , and type I-IFN will represent both natural IFN α , IFN ω , and IFN β .

Despite the fact that interferon is a potent antiviral agent, there is considerable evidence to suggest, that many of the characteristic symptoms of acute virus diseases such as upper respiratory tract infections are caused by an overproduction of interferon alpha. Furthermore, IFN alpha has been shown to contribute to the pathogenesis of certain chronic virus infections in experimental animals and the available evidence suggests that this is also the case for certain human chronic virus diseases such as those due to measles virus.

The interferons α are also potent immunoregulatory molecules which stimulate polyclonal B-cell activation, enhance NK cell cytotoxicity, inhibit T-cell functions, and modulate the expression of the major histocompatibility complex (MHC) class I antigens, all of which are implicated in the induction of autoimmunity and in graft rejection. The abnormal production of interferon α is associated with a number of autoimmune diseases and inflammatory disorders including systemic lupus erythematosus (SLE), type I diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, the acquired immunodeficiency syndrome (AIDS) and severe

combined immunodeficiency disease. The presence of interferon α in the serum of patients with systemic lupus is correlated with both the clinical and humoral signs of increased disease activity. The production of interferon α in HIV positive subjects is also highly predictive of disease evolution.

Administration of interferon α has been reported to exacerbate underlying disease in patients with psoriasis and multiple sclerosis and to induce a SLE like syndrome in patients without a previous history of autoimmune disease. Interferon α has also been shown to induce glomerulonephritis in normal mice and to accelerate the outset of the spontaneous autoimmune disease of NZB/W mice.

Interferon α is also produced during the course of graft-versus-host disease (GVHD) in parallel with the enhanced NK cell activity characteristic of systemic GVHD. Interferon α is the principal modulator of NK cell cytotoxicity and administration of interferon α has been shown to enhance the intestinal consequences of GVHD in normal mice.

The object of the present invention is to provide new antagonists against the biological activities of the human type I-IFN. These antagonists could be used for therapeutical, including prophylaxis purposes, in cases where the type I-IFN (IFN α/β) is abnormally produced and when this abnormal production is associated with pathological symptoms. Such antagonists could also be used for the diagnosis of various diseases or for the study of the evolution of such diseases.

In order to define such antagonists, the inventors have taken into account the fact that the human natural type I-IFN is in fact constituted of a mixture of

interferons (subspecies) and the fact that the composition of this association of different subtypes of interferons varies both quantitatively and qualitatively.

Some natural interferons, such as the ones secreted by Namalwa cells (Namalwa interferon) or leukocyte (leukocyte interferon) have been studied in detail (N.B. Finter and K.H. Fautes, Interferon 2, 1980, p. 65-79 I. Gresser Editor Academic Press ; K. Cantell et al, Interferon 1, 1979 p. 2-25, I. Gresser Editor Academic Press) and were used by the inventors to define natural type I interferons.

In some pathological cases, like AIDS, interferons having some special properties have been described (O.T. Preble et al, Annals of New-York Academy of Sciences p. 65-75). This interferon involved in pathological cases like AIDS nevertheless binds to the same receptor, as described above.

One object of the present invention is to provide an antagonist of the type I-IFN, which would be able to inhibit or neutralize, to a determined extent, the biological properties of the human type I-IFN, that is to say, to neutralize in vivo a mixture of α , β , ω subspecies.

Accordingly the inventors have defined antibodies, especially monoclonal antibodies, which have the property of being antagonists to the type I-IFN. These antibodies are directed against the human type I-IFN receptor.

The invention thus also concerns the use of the monoclonal antibodies for the preparation of pharmaceutical compositions, useful for the treatment of symptoms associated with the abnormal production of

type I-IFN. These monoclonal antibodies are also appropriate for the preparation of diagnosis reagents.

A monoclonal antibody according to the present invention is directed against the human type I-interferon receptor (IFN-R) and is characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

The ability to neutralize the biological properties of type I-IFN can be estimated as a function of the capacity of the monoclonal antibody to neutralize the antiviral activity of the type I-IFN. Such a test is relevant in order to determine whether the antibody assayed is included within the scope of the invention, although it is clear that the biological properties of type I-IFN are not limited to its antiviral properties. Detailed procedures are given in the examples in order to enable to perform such a test of the antiviral activity. The cells tested can advantageously be Daudi-cells, which affinity for the type I-IFN is well known. The main steps of such a test would consist in :

- incubating a determined concentration of human cells responsive to human type I-IFN, with human type I-IFN in the presence of a determined concentration of monoclonal antibodies to be assayed, for a time sufficient to allow the formation of a complex between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the incubated cells with a determined virus, in a determined concentration,
- washing the cells,
- resuspending the cells in culture medium,
- incubating for a time sufficient to allow virus replication ;
- lysing the cells ;
- measuring the virus replication, or measuring the inhibition of the cytopathic effect.

The ability of the monoclonal antibodies of the invention to neutralize the biological properties of the human type I-IFN can be modulated as a function of the dose of antibodies used. Accordingly a 100% inhibition of the biological properties, or a partial inhibition can be obtained.

According to another embodiment of the present invention, the monoclonal antibodies directed against the human type I-IFN receptor, are further characterized by the fact that they are capable of inhibiting the binding of a human type I-IFN, to the human IFN-R.

A monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN to its receptor, can be selected by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies or a hybridoma culture supernatant containing monoclonal antibodies to be assayed, with human cells capable of harboring IFN-R ;
- adding labelled human type I-IFN, in a determined concentration, to the above preincubated medium ;

- incubating the medium containing the human cells, the monoclonal antibodies and the labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the labelled type I-IFN by counting the amount of attached labelled type I-IFN.

Some of the monoclonal antibodies of the invention, have also the capacity to neutralize the antiproliferative properties of the human type I-IFN. This property can also be assayed on Daudi cells, by performing the following steps :

- allowing cells to grow in presence of human type IFN and determined concentration of mAb ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the human type I-IFN.

One property of a monoclonal antibody according to the invention resides in its capacity to recognize the extracellular domain of the human IFN receptor. This property of the monoclonal antibody can be assayed on human cells bearing the natural human receptor but also on the extracellular domain of a recombinant IFN-R such as expressed in a procaryotic cell, for instance in E.coli or a recombinant IFN-R such as expressed in a eucaryotic cell such as mamalian cell for instance a CHO-cell.

This receptor can indeed present different properties, depending on the fact that it is produced in a procaryotic or eucaryotic cell and accordingly

depending on the fact that the post-translational maturation occurred or not. The inventors interestingly showed that relevant assays, to evaluate the capacity of a monoclonal antibody according to the invention i.e. to recognize the cellular IFN-R, can be performed on a recombinant receptor expressed in mamalian cells. As a matter of fact, such recombinant receptor has the same properties as the cellular receptor, as far as its recognizing activity is concerned.

Monoclonal antibodies of the invention can be obtained against various forms of the receptor, including the complete receptor, a particular domain or a peptide characteristic of the aminoacid sequence of the receptor represented in figure 3.

Monoclonal antibodies of the invention can for example be prepared against the soluble form of the receptor. A hydrosoluble polypeptide corresponding to the soluble form of the INF-R is described on figure 2. According to the present invention, a soluble form of the IFN-R corresponds to a peptide or a polypeptide, capable of circulating in the body.

Other monoclonal antibodies according to the invention can also be prepared against a peptide comprised in the extracellular domain of the receptor as described on figure 2. An advantageous peptide corresponds for instance to the aminoacid sequence comprised between aminoacid 1 and aminoacid 229. According to another embodiment of the invention, the antibodies can be prepared against a polypeptide modified by substitution of one or more amino acids, provided that antibodies directed against the non modified extracellular domain of the IFN-R, recognize the modified polypeptide or peptide.

Preferred monoclonal antibodies according to the invention are those which are of the IgG1 type.

Among the antibodies of the invention, an antibody which has the capacity of inhibiting the binding of the type I-IFN to its receptor is preferably characterized in that it inhibits the in vitro binding of human type IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0.5 to 2 $\mu\text{g/ml}$.

The inventors have shown that the high affinity binding capacity of a monoclonal antibody is not sufficient to ensure that this antibody will be able to inhibit the binding activity of the human type I-IFN to the IFN-R. Nevertheless the high affinity binding capacity of the monoclonal antibody is necessary to investigate further the ability of the antibody to inhibit the binding of the type I-IFN to its cellular receptor.

Another monoclonal antibody is characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

According to another embodiment a monoclonal antibody is also characterized in that it neutralizes in vitro the antiproliferative activity of human type IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

A particular group of monoclonal antibodies according to the invention is characterized in that it

neutralizes the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 50 $\mu\text{g/ml}$, preferably 1 to 20 $\mu\text{g/ml}$, for a concentration of type I-IFN in the range of 1 to 1000 units with reference to the international standard MRC 69/19.

Advantageously, the monoclonal antibody according to the invention is such that these antibodies do not bind to the human receptor for IFN gamma.

One particular antibody satisfying the requirements of the invention, is such as it directed against an epitope on the amino-acid sequence comprised between amino-acid 27 and amino-acid 427 of the extracellular domain of the human IFN-R as represented on figure 2.

One particularly interesting monoclonal antibody according to the invention is the antibody designated 64G12 under n° 92022605 which has been deposited at the ECACC (European Collection of Animal Cell Cultures Porton Down Salisbury, Wiltshire SP4 056, United Kingdom) on February 26, 1992.

These antibodies may be prepared by conventional methods involving the preparation of hybridoma cells by the fusion of myeloma cells and spleen cells of an animal immunized beforehand with the peptide antigen, on the conditions such that the antigen against which the antibodies are formed is constituted by the extracellular domain of IFN-R or any polypeptide or peptide of this domain.

The hybridomas are constructed according to the protocole of Kohler and Milstein (Nature, 1974, 256: 495-497). For example the hybridomas are derived from

the fusion of the spleen cells above described with NS1 mouse (BalbC) HGPRT⁻ as myeloma cell.

A second procedure for the production of monoclonal antibodies according to the invention, consists in carrying out the fusion between B-cells of blood immortalized with the Epstein/Barr virus and human B lymphocytes placed beforehand in contact with the extracellular domain or a fragment thereof of the IFN-R, against which it is decided to form monoclonal antibodies. B-cells placed in contact beforehand with the extracellular domain of IFN-R or fragment thereof against which it is decided to form monoclonal antibodies, may be obtained by in vitro culture contacted with the antigens, the recovery of the B-cells coated with these antigens being preceded by one or several cycles of stimulation.

The invention thus concerns human antibodies as obtained by carrying out the above procedure, having the above defined properties.

The invention also aims at providing a monoclonal antibody characterized in that the variable or complementary determining regions of its heavy and/or light chains are grafted on the framework and/or constant regions of a human antibody.

The invention further provides a composition having antagonist properties for the biological properties of the human type I-IFN, characterized in that it comprises monoclonal antibodies as defined above.

Accordingly the invention provides a pharmaceutical composition characterized in that it comprises monoclonal antibodies as defined above, together with an appropriate pharmaceutical vehicle.

The invention also concerns the use of a monoclonal antibody as defined above, for the manufacture of a drug for the treatment or profilaxis of a pathological state or symptoms associated with overproduction of type-I-IFN.

According to a first example, the antibodies can be used in a pharmaceutical composition, for the treatment of allograft rejection.

According to another example, antibodies of the invention are used as active principle in a pharmaceutical composition for the treatment of autoimmune and inflammatory diseases. Such diseases include systemic lupus erythematosus, type 1 diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, acquired immunodeficiency syndrome (AIDS), and severe combined immunodeficiency disease.

Treatment of acute virus diseases can also be performed with the antibodies of the invention. As example upper respiratory tract infections, chronic virus infections such as those due to measles virus, can be performed.

The antibodies of the invention can also be used for the in vitro diagnosis of the presence of the human type I-IFN receptor or cells.

Further details and additional information will arise from the description from the description of the examples and from the figures.

FIGURES

- Figure 1 : binding of ^{125}I -labelled monoclonal antibodies 34F10 and 64G12 to :

- A : Daudi cells
- B : Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was measured by incubation with a 100 fold excess of cold antibodies and subtracted from total counts.

- Figure 2 : nucleotide and corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthesized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in figure 2.

- Figure 3 : nucleotide and corresponding amino-acid sequence of the human IFN-R.

EXAMPLES**EXAMPLE 1 :**Synthesis of the soluble receptorsSynthesis in E.coli

A fragment of DNA containing the sequence coding for the extracellular domain (amino acids 27 to 427) of the human INF-R (figure 2), in which an extra-sequence coding for 5 histidyl residues was introduced just before the termination codon, was cloned in the expression vectors pKK233-2. This fragment was produced by the Polymerase Chain Reaction (PCR) and the resulting plasmids were sequenced to confirm both in-frame insertion with the Shine-Dalgarno sequence and the appropriate sequence coding for the receptor.

The poly-histidyl tail introduced into the recombinant protein enables it to be purified rapidly by affinity chromatography on a chelated nickel support (NTA column) as described previously (Hochuli E. et al, Bio/technology, 1988, 1321-1325).

The plasmid was introduced into the E.coli strain, JM105, and protein synthesis induced by addition of IPTG to the culture medium (pKK233-2, tac promoter).

Proteins were extracted from the bacterial pellet and the soluble receptor purified to homogeneity by affinity chromatography as described hereafter. This procedure yielded a protein that migrates as 2 bands around 50 kDa under reducing conditions and three bands under non-reducing conditions. The maximum concentration of the protein obtained by different procedures was approximately 20µg/ml.

The N-terminal sequence of the two proteins detected by gel electrophoresis has shown that both proteins are the expected fragment of the receptor.

Synthesis and purification of an unglycosylated soluble receptor :

Bacterial culture (250ml)

|

IPTG induction 3h

|

cell pellet

6M Guanidine hydrochloride pH8

|

centrifugation

|

NTA column:

Washes pH 8 urea 8M

pH 6,3 urea 8M

pH 5.9 urea 8M

|

Elution pH 4 urea 8M

|

refolding dilution, dialysis

|

against Tris 0,1 M pH9

dialysis PBS

Using the same PCR approach, we also constructed an expression vector coding for the IFN-R amino acid sequence 1-427, with an additional 5-histidyl residues at the C-terminus, inserted in expression vector pXMT-3. The exact nucleotide sequence of the insert was also confirmed.

The resulting plasmid was introduced by electroporation into Cos7 cells for transient expression and the recombinant protein was purified to homogeneity by affinity chromatography followed by ion exchange chromatography on mono-Q (Pharmacia) as described hereafter.

Purification of the soluble IFN-R from Cos7 cells

preparative electroporation of
cos cells

| 18 h
|

serum free medium
|

supernatants taken after 48h, 72h, 96h
|

concentration
|

NTA column
|

Wash PBS

elution 0.1 M NaOAc pH 5.5
|

neutralization

concentration, 30 000 cut off
|

Mono Q (0-0.5 M Na Cl)

This purification yielded to a 76 kDa protein whose N-terminal sequence corresponds to the predicted receptor sequence with some heterogeneity in the processing of the leader sequence.

EXAMPLE 2 :

Production of monoclonal antibodies against the interferon type I receptor

1) Production of the monoclonal antibodies

Mice were immunized by injection of recombinant soluble interferon (r sIFN-R) purified from E.coli or from a culture supernatant of Cos7 cells. Initially mice were injected both intraperitoneally and subcutaneously with the purified protein in complete Freund's adjuvant. Subsequently mice were injected once a week intraperitoneally with the purified proteins diluted in buffered saline solution. Ten micrograms of recombinant proteins were injected each time.

After the fourth injection, blood was collected and the presence of specific serum antibodies were tested by both ELISA and Western blot against the recombinant receptor. The strongest responders were then boosted with a total of 10 μ g of antigen half of which was injected intravenously and half intraperitoneally.

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT⁻ myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35:1-32, 1980). Briefly, 5×10^7 spleen cells were fused to 3×10^7 myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as 20×10^7 spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method :

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.05% Tween 20,

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% Tween 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

3) Identification of reactivity to the natural interferon type I receptor

The reactivity of the monoclonal antibodies (mAbs) recognizing the recombinant sIFN-R was tested against the natural class I receptor expressed at the surface of Daudi cells, by membrane immunofluorescence. Briefly, 5×10^5 Daudi cells were incubated in 100 μ l of culture supernatant of chosen hybridomas for 30 min at 4°C. The cells were then washed 4 times in RPMI medium containing 1% BSA and further incubated with a diluted FITC labelled goat anti-mouse F(ab')₂ for 30 min at 4°C. The cells were finally analyzed by flow cytometry after washing. One of the 35 tested antibodies produced against the E.coli recombinant receptor and 5 of the 6 tested antibodies produced against the COS recombinant receptor were found to recognize the natural receptor on the Daudi cells.

Cloning of these hybridomas was then performed by limiting dilution. The isotype of these mAbs was determined by an ELISA method using isotype specific antibodies. All 6 mAbs were found to be IgG1 with kappa light chains. A summary of the reactivity of these 6 mAbs is given in Table 1.

Monoclonal antibodies were purified from culture supernatants by protein G chromatography.

Table 1 :**Reactivity of the anti IFN-R monoclonal antibodies**

	Reactivity against the recombinant receptor				Reactivity against * the cellular receptor
	E.COLI		COS		immunofluorescence
	ELISA	Western	ELISA	Western	
34F10	+	+	+	+	+
64G12	+	+	+	+	+
63F6 64G2 64D10 65D8	-	-	+	+	+
				weak	

* measured on Daudi cells

EXAMPLE 3 :**Inhibition of the binding of interferon to human cell lines**

Inhibition of interferon binding to human cells was assayed as follows. 10^6 cells were preincubated at 4°C for 30 min with various dilutions of hybridoma culture supernatants or purified mAbs or with medium alone. ^{125}I -labelled IFN alpha 8 or alpha 2 was added at the concentration of 100pM and cells incubated for a further 2 hours at 4°C. These incubations were performed in RPMI medium containing 20mM HEPES pH 7.4 and 10% foetal calf serum (FCS). The cells were finally washed 4 times with RPMI - 1% FCS and counted to determine bound radioactivity.

The mAb secreted by the hybridoma line 64G12 (latter named mAb 64G12) was shown in this assay to inhibit the binding of labelled IFN to the cells in a dose-dependent manner. 50% inhibition of binding to the Daudi cells (Burkitt lymphoma cell line ; Klein et al., Cancer Research, 28:1300-1310, 1968) was obtained at a mAb concentration of 0.4 μ g/ml. The same inhibition was obtained with K562 cells (chronic myelogenous leukemia, Lozzio and Lozzio, Cell, 45:321-334, 1975) but 50% inhibition was obtained at 11 μ g/ml for HL60 cells (Promyelocytic leukemia, Collins S.J. et al., Nature, 270:347-349, 1977) and 60 μ g/ml for Ly28 cells (Klein G. et al. Int. J. Cancer, 10:44-57, 1972).

Table 2 :

The inhibition of binding of labelled IFN alpha 2 to various cell lines by mAb64G12

Cell lines	Concentration of mAB which gives 50% inhibition of binding
Daudi K562	0,4 μ g/ml
HL60	11 μ g/ml
Ly28	60 μ g/ml

The difference in the mAb concentration at which 50% inhibition of binding of IFN is obtained has been investigated by direct binding of ¹²⁵I-labelled mABs 64G12 and 34F10 to the same cell lines and Scatchard

plot analysis of the results. In the concentration range of 0.1 to 1.5 $\mu\text{g/ml}$, a high affinity binding of the mAb 34F10 ($\approx 10\text{nM}$) was seen on all cell lines whereas a high affinity binding of mAb 64G12 was only detected on Daudi and K562 cells (Figure 1).

EXAMPLE 4 :

Inhibition of the function of type I interferon

Functional inhibition of type I interferon by the purified mAb 64G12 was demonstrated in an antiviral assay on Daudi cells using either recombinant IFN alpha 2, IFN beta and IFN omega, or purified Namalwa and leucocyte interferons, and in an antiproliferative assay with recombinant IFN alpha 2.

* Antiviral activity

An antiviral assay on Daudi cells was performed as described (M. Dron and M.G. Tovey, J. Gen. Virol. 64:2641-2647, 1983). Cells ($0.5 \times 10^6/\text{ml}$) were incubated for 24 hours in the presence of interferon and antibodies. 10^6 cells in 1 ml were then infected for 1 hour at 37°C with Vesicular stomatitis virus (VSV) then washed 3 times, resuspended in culture medium and incubated for 18 hours at 37°C . Cells were then lysed by freeze-thawing and virus replication measured by titration of the supernatants on L929 cells. A dose-dependent inhibition of the antiviral activity of the various subtypes of type I IFN was demonstrated for the purified mAb 64G12.

For the antiviral assay with the Wish cells, cells were incubated for 24 hours with various concentrations of interferons in the presence of the mAbs prior to challenge with VSV. In this assay, the mAb 64G12 was demonstrated to block completely the antiviral activity of Leukocyte IFN (50U/ml), recombinant IFN alpha 2 (50U/ml) and interferon from the sera of AIDS patients (50, 75 and 150U/ml).

* antiproliferative activity

For the antiproliferative assay, Daudi cells were seeded at a concentration of 10^5 cells per ml in a 96 well plate in the presence of interferon and purified inhibitory or control antibody. Cells were then counted after 24, 48 and 72 hours with a Coulter counter and checked for viability by trypan blue exclusion. Purified mAb 64G12 demonstrated a dose-dependent inhibition of the antiproliferative activity of interferon alpha 2.

C L A I M S

1. Monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

2. Monoclonal antibody directed against the human type I IFN-R according to claim 1, characterized by its capacity to inhibit the binding of a human pathological type I-IFN, to the IFN-R.

3. Monoclonal antibody according to claim 1 or 2, which is obtainable from a hybridoma cell prepared by fusion of a myeloma cell with spleen cells from an animal previously immunized with the soluble form of the human IFN-R.

4. Monoclonal antibody according to anyone of claims 1, 2 or 3, characterized in that it recognizes an epitope on a soluble form of the human cellular IFN-R or of a recombinant IFN-R.

5. Monoclonal antibody according to anyone of claims 1 to 4, characterized in that it inhibits in vitro the binding of human type I-IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0,5 to 2 $\mu\text{g/ml}$.

6. Monoclonal antibody according to anyone of claims 1 to 5, characterized in that it neutralizes in vitro the antiproliferative activity of the human type I-IFN, on cells highly responsive to this human type I-IFN,

for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

7. Monoclonal antibody according to anyone of claims 1 to 6, characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells poorly responsive to this human type I-IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

8. Monoclonal antibody according to anyone of claims 1 to 7, characterized in that it does not bind to the human receptor of the IFN gamma.

9. Monoclonal antibody according to anyone of claims 1 to 8, characterized in that it recognizes an epitope on the aminoacid sequence 27 to 427 of the human IFN-R.

10. Monoclonal antibody according to anyone of claims 1 to 9, characterized in that it neutralizes in vitro the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

11. Monoclonal antibody according to anyone of claims 1 to 10, characterized in that it neutralizes in vitro the antiviral activity of the human class I-IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

12. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is the 64G12 antibody, deposited at the ECACC on February 26, 1992 under n° 92022605.

13. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a humanized antibody, for instance characterized in that the variable or complementary determining regions of its

heavy and light chains are grafted on the framework and constant regions of a human antibody.

14. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a human antibody.

15. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is an IgG1 type antibody.

16. Hybridoma cell, characterized in that it produces monoclonal antibodies according to claims 1 to 13.

17. Composition having antagonist properties to the type I-IFN, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 16.

18. Pharmaceutical composition, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 17, together with an appropriate pharmaceutical vehicle.

19. Use of a monoclonal antibody according to anyone of claims 1 to 17, for the manufacture of a drug for the treatment or prophylaxis of a pathological state associated with proliferative cell activity and/or viral cell infection.

20. Process for the selection of a monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN, to the IFN-R, characterized by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies according to anyone of claims 1 to 15 or a hybridoma culture supernatant containing monoclonal antibodies, with human cells susceptible of harboring IFN-R ;
- adding labelled human type I-IFN in a determined concentration, to the above preincubating medium ;

- incubating the medium containing the human cells, monoclonal antibodies and labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the type I-IFN, by counting the amount of attached labelled type I-IFN.

21. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiproliferative activities of the type I-IFN, on human cells characterized by the steps of :

- allowing cells to grow in the presence of human type I-IFN and in the presence of a determined concentration of monoclonal antibodies according to anyone of claims 1 to 15 ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the type I-IFN.

22. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiviral activities of the natural, non pathological or pathological type I-IFN on human cells, characterized by the steps of :

- incubating cells with type I-IFN and monoclonal antibodies according to anyone of claims 1 to 15, in determined concentrations, for a time sufficient to allow the formation of a complex

between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the above incubated cells with a determined concentration of a virus ;
- washing the cells ;
- resuspending the cells in culture medium ;
- incubating for a time sufficient to allow the replication of the virus ;
- lysing the cells and ;
- measuring the virus replication or measuring the inhibition of the cytopathic effect.

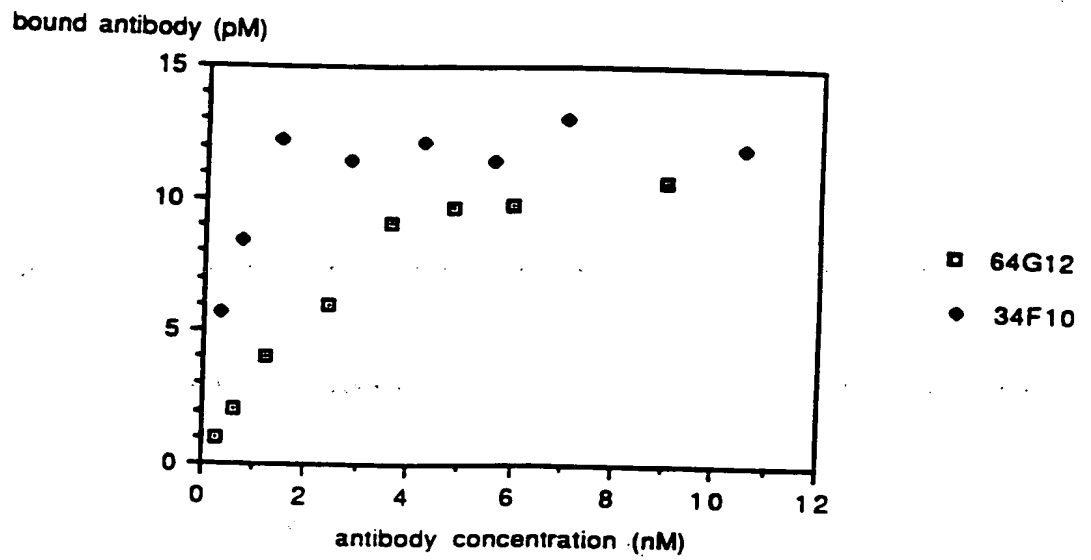
A B R I D G M E N TMONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The invention relates to a monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

It further concerns their use for the diagnosis.

A



B

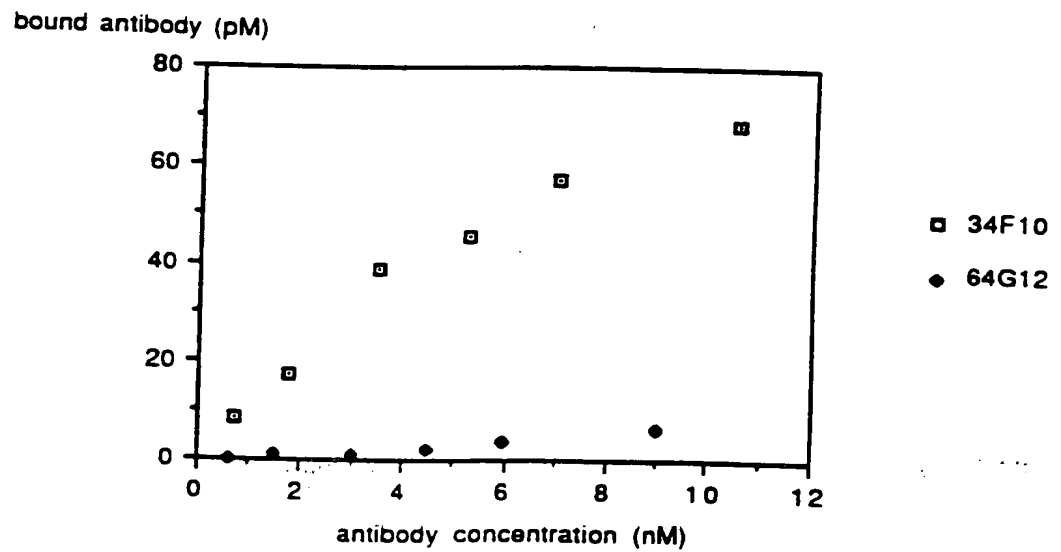


FIGURE 1

CTGCAGGGATCTGCGGCGGCTCCCAG

ATG ATG GTC GTC GTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 2A

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn MET Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu

AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr MET Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA TGAGGTACC
Asn Thr Ser Lys

1334

FIGURE 2B

CTGCAGGGATCTGCCGGCGCTCCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTC ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 3A

ATT GAA AAT ATA AGC ACA ATT GCT ACA GTA GAA GAA ACT AAT CAA ACT GAT GAA
 Ile Glu Asn Ile Ser Thr Ile Ala Thr Val Glu Glu Thr Asn Gln Thr Asp Glu

GAT CAT AAA AAA TAC AGT TCC CAA ACT AGC CAA GAT TCA GGA AAT TAT TCT AAT
 Asp His Lys Lys Tyr Ser Ser Gln Thr Ser Gln Asp Ser Gly Asn Tyr Ser Asn

GAA GAT GAA AGC GAA AGT AAA ACA AGT GAA GAA CTA CAG CAG GAC TTT GTA TGA
 Glu Asp Glu Ser Glu Ser Lys Thr Ser Glu Glu Leu Gln Gln Asp Phe Val

CCAGAAATGAACTGTGTCAAGTATAAGGTTTTTCAGCAGGAGTTACACTGGTACC

1697

FIGURE 3C

LA POSTE

AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDÉ

RA 6156 2078 9FR

Présenté le : 13/12/01

Distribué le :

Signature du destinataire :

~~NON REÇU
RETOUR À L'ÉMETTEUR~~

RETOUR À :

GUTMANN, PLASSERAUD
3 Rue 114ème - Vanvres
75008 PARIS

SIREN 666 000 000 RCS NANTERRE

UTILISER UN STYLO À BILLE / APPUYER

SIREN 666 000 000 RCS NANTERRE

AVIS DE RÉCEPTION

LETTRE

Bureau de :

PARIS TOUSIEUX
30 bis, rue du Cardinal Lemoine
75231 PARIS CEDEX 05

REÇU LE

06 AOUT 2001

E. GUTMANN - Y. PLASSERAUD S.A.



BDS
Unknown
Client Number

17283/123

ANNEX 19

Translation from French into English of the back of the envelope mentioned in Annex 18.

Annex 19: Non-collected mail

Acknowledgement of Receipt
of your registered mail

Not asked for
Return to the expediter

LA POSTE

AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDE

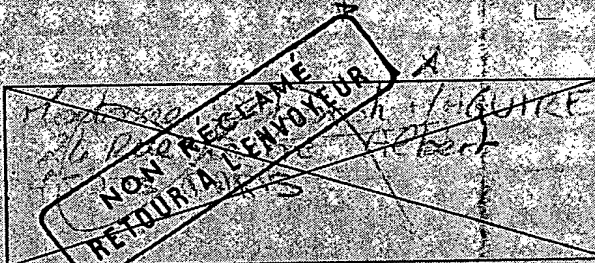
RA 6156 2078 9FR

JACQUINIA 2100FACH

Présenté le : 13/02/01

Distribué le :

Signature du destinataire



RETOUR A :

GUTMANN - PLASSERAUD
3 Rue Chevreu - Loppet
75008 PARIS

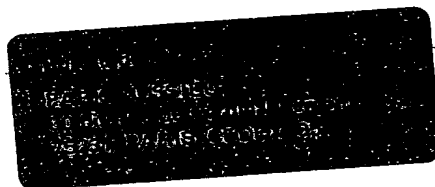
BIEN 356 000 000 RCS NANTERRE

AVIS DE RÉCEPTION

BIEN 356 000 000 RCS NANTERRE

UTILISER UN STYLO A BILLE / APPUYER

LETTRE



Office where the mail
was stacked



REÇU LE
06 AOUT 2001
E. GUTMANN - Y. PLASSERAUD S.A.

Stamp showing the date
when the mail was received
after it was returned
to E. Gutmann - Y. Plasseraud S.A.



Stamp showing that the mail was
still in the post office on August 3, 2001

VERIFICATION OF TRANSLATION

I, Julia ANDRAL-ZIURYS, working at ERNEST GUTMANN-YVES PLASSERAUD S.A., 3 rue Chauveau-Lagarde, 75008 Paris (France),

declare that I am conversant with the French and English languages and that to the best of my knowledge and belief the following (Annex 19) is a true translation of the indications on the back of the envelope of our registered mail of July 16, 2001 to Mrs. MAGUIRE that was not collected by the latter and returned to us (Annex 18).

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of this application or any patent issuing thereon.

Paris, November 22, 2001

Signature:


21/11/01
Julia ANDRAL-ZIURYS

ANNEX 20

2nd sending of the same package to Ms. MAGUIRE on September 4, 2001, with the same letter and the same enclosures. This package was returned to us on September 25, 2001, for the same reason as the first one, i.e., it was not collected.



ERNEST GUTMANN - YVES PLASSERAUD S.A.
SOCIETE DE CONSEILS EN PROPRIETE INDUSTRIELLE

PARIS (SIÈGE) :
3, RUE CHAUVEAU-LAGARDE
F - 75008 PARIS
TÉL. : 33 (0)1 44 51 18 00
FAX : 33 (0)1 42 66 08 90
e-mail : info@egyp.fr

LYON (AGENCE) :
62, RUE DE BONNEL
F - 69448 LYON CEDEX 03
TÉL. : 33 (0)4 72 84 97 60
FAX : 33 (0)4 72 84 97 65

ALICANTE (AGENCE) :
PLAZA DEL AYUNTAMIENTO 2-2º-2º
ES - 03002 ALICANTE
TÉL. : 34 965 230 611
FAX : 34 965 230 639

BDS 17283/123

Par RECOMMANDE A/R

Madame Deborah MAGUIRE
24 rue Maître-Albert

75005 PARIS

2^e envoi

VOIRE REFERENCE :

NOTRE REFERENCE :

LE

B1608ACA – JAZ/VMA/MNH

04 septembre 2001

Objet : Brevet américain n° US 5,919,453 du 6 juillet 1999
Demande divisionnaire n° 09/240,675 déposée le 2 février 1999
au nom de MEDISUP INTERNATIONAL N.V.
Inventeurs : BENOIT, MEYER, MAGUIRE, PLAVEC, TOVEY

BREVETS

Ernest GUTMANN, cpi *
Anne DESAIX, cpi * °
Carol ALMOND-MARTIN * (1)
Julia ANDRAL-ZIURYS °
Florence LAZARD, cpi
Jeanne VAILLANT, cpi * °
Véronique MARCADÉ
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Carole SELLIN (1)

MARQUES, DESSINS
ET MODÈLES

Yves PLASSERAUD, cpi * °
Martine DEHAUT, cpi °
Virginie ZANCAN, cpi °
Nathalie PACAUD
Benjamin FONTAINE (2)
Christophe PELÈSE

DOCUMENTATION
ET VEILLE TECHNOLOGIQUE

Jean-Charles THEODET

Chère Madame,

En tant qu'inventeur désigné dans le brevet américain cité en référence, vous avez signé, le 11 novembre 1994, une déclaration donnant pouvoir à nos correspondants de la société Foley & Lardner, afin qu'ils vous représentent auprès de l'Office Américain des Brevets. Une copie de cette déclaration est ci-jointe.

Dans le cadre d'une demande de brevet divisionnaire, portant sur des peptides particuliers reconnus par l'anticorps monoclonal 64G12, l'Examinateur américain en charge de ce dossier considère que cette déclaration est défectueuse car certaines corrections manuscrites y ont été apportées sans être datées et paraphées. Il nous demande donc de lui fournir une nouvelle déclaration.

Vous trouverez donc ci-joint, une copie du texte initialement déposé ainsi qu'une copie des revendications de la demande divisionnaire. Vous trouverez aussi un exemplaire d'une déclaration supplémentaire, que nous vous prions de bien vouloir nous renvoyer après l'avoir datée et signée. Nous attirons votre attention sur le contenu du sixième paragraphe de cette déclaration, qui précise que vous avez lu et compris la description et l'ensemble des revendications ci-jointes. Si vous avez la moindre question à ce sujet, n'hésitez pas à nous contacter. Par ailleurs, si vous

mandataire agréé OEB/EPO
° US patent attorney
° conseil européen en marques
OHM/OHIM


(1) Agence de Lyon
(2) Agence d'Alicante

SOCIETE ANONYME
AU CAPITAL DE 3 000 000 F
RCS PARIS B 332 417 500
APE 741 A

Acquisition et défense des droits, stratégie de protection, liberté d'exploitation
et recherches de disponibilité, oppositions, consultations, contrats et audits

décidez de ne pas signer cette déclaration, nous vous remercions de bien vouloir nous en avertir.

Veuillez agréer, Chère Madame, l'expression de nos meilleures salutations.



Véronique MARCADÉ



Julia ANDRAL-ZIURYS

- P.J. : - Déclaration du 11/11/94
- Texte initialement déposé et revendications de la demande divisionnaire
- Déclaration supplémentaire

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR,
WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

(Attorney Docket No. 017283/0123)

the specification of which (check one)

☐ Is attached hereto.

☒ Was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
92400902.0	European	31/March/1992	Yes	NO

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

STEPHEN A. BENT	Reg. No. 29,768
DAVID A. BLUMENTHAL	Reg. No. 26,257
BETH A. BURROUS	Reg. No. 35,087
ALAN I. CANTOR	Reg. No. 28,163
WILLIAM T. ELLIS	Reg. No. 26,874
JOHN J. FELDHAUS	R g. No. 28,822
MICHAEL D. KAMINSKI	R g. No. 32,904
LYLE K. KIMMS	R g. No. 34,079

KENNETH E. KROSIN	Reg. No. 25,735
JOHNNY A. KUMAR	R g. No. 34,649
JACK LAHR	R g. No. 19,621
GLENN LAW	Reg. No. 34,371
PETER G. MACK	Reg. No. 26,001
STEPHEN B. MAEBIUS	Reg. No. 35,264
BRIAN J. MC NAMARA	Reg. No. 32,789
SYBIL MELOY	Reg. No. 22,749
RICHARD C. PEET	Reg. No. 35,792
GEORGE E. QUILLIN	Reg. No. 32,792
ANDREW E. RAWLINS	Reg. No. 34,702
BERNHARD D. SAXE	Reg. No. 28,665
CHARLES F. SCHILL	Reg. No. 27,590
RICHARD L. SCHWAAB	Reg. No. 25,479
MICHELE M. SIMKIN	Reg. No. 34,717
HAROLD C. WEGNER	Reg. No. 25,258

to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

I request that all correspondence be directed to:

Bernhard D. Saxe
Foley & Lardner
At Washington Harbour
3000 K Street, NW, Suite 500
Washington, DC 2000

Telephone: 202-672-5472
Facsimile: 202-672-5399

I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Name of first inventor	<u>Patrick BENOIT</u>
Residence	<u>Paris, France</u>
Citizenship	<u>FRANCE</u>
Post Office Address	<u>24, rue Jonquoy, F-75014 Paris, France</u>
Inventor's signature	<u></u>
Date	<u></u>

Name of second inventor	<i>Francois MEYER</i>
Residence	<i>Paris, France</i>
Citizenship	<i>LUXEMBOURG</i>
Post Office Address	<i>3 Place du Panthéon, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of third inventor	<i>Deborah MAGUIRE</i>
Residence	<i>Paris, France</i>
Citizenship	<i>AUSTRALIA</i>
Post Office Address	<i>24, rue Maitre-Albert, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of fourth inventor	<i>Ivan PLAVEC</i>
Residence	<i>Sunnyvale, CA, USA</i>
Citizenship	<i>CROATIA</i>
Post Office Address	<i>1415, Mallard Way, Sunnyvale, CA 94087, USA</i>
Inventor's signature	
Date	
Name of fifth inventor	<i>Michael G. TOVEY</i>
Residence	<i>Paris, France</i>
Citizenship	<i>GREAT BRITAIN</i>
Post Office Address	<i>7, rue Lagrange, F-75005 Paris, France</i>
Inventor's signature	
Date	

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:
MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE 1 INTERFERON
the specification of which (check one)

☐ is attached hereto

☒ was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before the date of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
92400902.0	European	31/March/1992	Yes

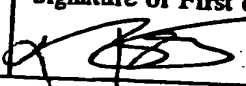
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; John J. Feldhaus, Reg. No. 28,822; Donald D. Jeffery, Reg. No. 19,980; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Neil Meloy, Reg. No. 22,749; George E. Quillen, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 22,665; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115.

Send all correspondence to **FOLEY & LARDNER**, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696.
Address telephone communications to Bernhard D. Saxe at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor Patrick BENOIT		Signature of First or Sole Inventor 	Date 11/11/94
Residence Address Paris, France		Country of Citizenship FRANCE	
Post Office Address 24, rue Jonquoy, F-75014 Paris, France			

Signatures should conform to names as typewritten. ☒ Additional inventors on attached Page 2.

Full Name of Second Inventor Francois MEYER	Signature of Second Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 14. square Adanson. F-75005 Paris, France		

Deborah

Full Name of Third Inventor Deborah-MAGUIRE	Signature of Third Inventor <i>[Signature]</i>	Date 11/14/94
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 24, rue Maitre-Albert. F-75005 Paris, France		

Full Name of Fourth Inventor Ivan PLAVEC	Signature of Fourth Inventor <i>[Signature]</i>	Date 11/14/94
Residence Address Fresnes, France	Country of Citizenship FRANCE CROATIA	
Post Office Address 1.allee du Capitaine-Dupont F-92260 Fresnes, France		

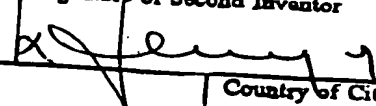
Full Name of Fifth Inventor Michael G. TOVEY	Signature of Fifth Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
Residence Address Paris, France	Country of Citizenship GREAT BRITAIN	
Post Office Address 6, rue des Quatrefoies, F-75005 Paris, France		


Full Name of Second Inventor Francois MEYER	Signature of Second Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 14, square Adanson, F-75005 Paris, France		

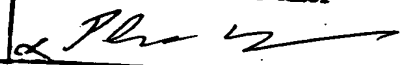
Full Name of Third Inventor Deborah MAGUIRE	Signature of Third Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
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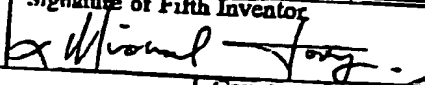
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*Revendication de la demande
divisionnaire*

IN THE CLAIMS:

Please delete claims 1-22 and insert the following new claims:

--23. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 27 to amino acid residue 427 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12 (deposited at the ECACC under no. 92022605). ---

24. A peptide or polypeptide as claimed in claim 23, consisting of amino acid residue 27 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof.

25. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 1 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12.

26. An analogue of a peptide or polypeptide as claimed in claim 23, which is derived from said peptide or polypeptide by substitution of one or more amino acid residues and which retains the ability to specifically bind to monoclonal antibody 64G12.

27. A method of producing a monoclonal antibody, comprising immunizing an animal with a peptide or polypeptide as claimed in claim 23, fusing spleen cell from the immunized animal with myeloma cells, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide.

28. A method of producing a monoclonal antibody, comprising contacting stimulated B-lymphocytes *in vitro* with a peptide or polypeptide according to claim 23, fusing the resultant B-lymphocytes with B-lymphocytes immortalized with Epstein-Barr

virus, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide. --

IN THE ABSTRACT

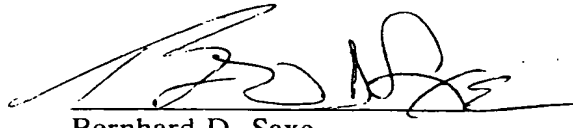
Please insert the Abstract provided on the attached sheet.

REMARKS

The Examiner is respectfully requested to enter the above amendments prior to examination of the instant application. Support for the amendments is present throughout the specification, in particular at pages 10-11.

Respectfully submitted,

February 2, 1999
Date


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ABSTRACT OF THE DISCLOSURE

A monoclonal antibody is provided which is directed against the human interferon type I receptor (IFN-R), which recognizes the extracellular domain of the human IFN-R and which has neutralizing capacity against the biological properties of human type I-IFN. Diagnostic and therapeutic applications for the monoclonal antibody also are provided.

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN : alpha (α), beta (β) and gamma. IFN α and IFN β together are known as the type I interferon.

Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33:251, 1986).

The human IFN α locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, α II or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFN α genes (Weissmann and Weber 1986).

The subtypes of IFN α have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78:2848, 1981) and have the same cellular receptor (Agnet M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFN β) is encoded by a single gene which has approximately 50% homology with the IFN α genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

The interferon gamma (IFN gamma) is also encoded by a single copy, which has little homology with the IFN α and IFN β genes. The receptor for IFN gamma is distinct from the receptor of the α and β interferons.

For the purpose of the present invention the receptor of α and β classes of IFN will be designated IFN-R. This represents natural type I receptor. The group of proteins forming natural interferon α will be designated IFN α , and type I-IFN will represent both natural IFN α , IFN ω , and IFN β .

Despite the fact that interferon is a potent antiviral agent, there is considerable evidence to suggest, that many of the characteristic symptoms of acute virus diseases such as upper respiratory tract infections are caused by an overproduction of interferon alpha. Furthermore, IFN alpha has been shown to contribute to the pathogenesis of certain chronic virus infections in experimental animals and the available evidence suggests that this is also the case for certain human chronic virus diseases such as those due to measles virus.

The interferons α are also potent immuno-regulatory molecules which stimulate polyclonal B-cell activation, enhance NK cell cytotoxicity, inhibit T-cell functions, and modulate the expression of the major histocompatibility complex (MHC) class 1 antigens, all of which are implicated in the induction of autoimmunity and in graft rejection. The abnormal production of interferon α is associated with a number of autoimmune diseases and inflammatory disorders including systemic lupus erythematosus (SLE), type I diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, the acquired immunodeficiency syndrome (AIDS) and severe

combined immunodeficiency disease. The presence of interferon α in the serum of patients with systemic lupus is correlated with both the clinical and humoral signs of increased disease activity. The production of interferon α in HIV positive subjects is also highly predictive of disease evolution.

Administration of interferon α has been reported to exacerbate underlying disease in patients with psoriasis and multiple sclerosis and to induce a SLE like syndrome in patients without a previous history of autoimmune disease. Interferon α has also been shown to induce glomerulonephritis in normal mice and to accelerate the outset of the spontaneous autoimmune disease of NZB/W mice.

Interferon α is also produced during the course of graft-versus-host disease (GVHD) in parallel with the enhanced NK cell activity characteristic of systemic GVHD. Interferon α is the principal modulator of NK cell cytotoxicity and administration of interferon α has been shown to enhance the intestinal consequences of GVHD in normal mice.

The object of the present invention is to provide new antagonists against the biological activities of the human type I-IFN. These antagonists could be used for therapeutical, including prophylaxis purposes, in cases where the type I-IFN (IFN α/β) is abnormally produced and when this abnormal production is associated with pathological symptoms. Such antagonists could also be used for the diagnosis of various diseases or for the study of the evolution of such diseases.

In order to define such antagonists, the inventors have taken into account the fact that the human natural type I-IFN is in fact constituted of a mixture of

interferons (subspecies) and the fact that the composition of this association of different subtypes of interferons varies both quantitatively and qualitatively.

Some natural interferons, such as the ones secreted by Namalwa cells (Namalwa interferon) or leukocyte (leukocyte interferon) have been studied in detail (N.B. Finter and K.H. Fautes, Interferon 2, 1980, p. 65-79 I. Gresser Editor Academic Press ; K. Cantell et al, Interferon 1, 1979 p. 2-25, I. Gresser Editor Academic Press) and were used by the inventors to define natural type I interferons.

In some pathological cases, like AIDS, interferons having some special properties have been described (O.T. Preble et al, Annals of New-York Academy of Sciences p. 65-75). This interferon involved in pathological cases like AIDS nevertheless binds to the same receptor, as described above.

One object of the present invention is to provide an antagonist of the type I-IFN, which would be able to inhibit or neutralize, to a determined extent, the biological properties of the human type I-IFN, that is to say, to neutralize in vivo a mixture of α , β , ω subspecies.

Accordingly the inventors have defined antibodies, especially monoclonal antibodies, which have the property of being antagonists to the type I-IFN. These antibodies are directed against the human type I-IFN receptor.

The invention thus also concerns the use of the monoclonal antibodies for the preparation of pharmaceutical compositions, useful for the treatment of symptoms associated with the abnormal production of

type I-IFN. These monoclonal antibodies are also appropriate for the preparation of diagnosis reagents.

A monoclonal antibody according to the present invention is directed against the human type I-interferon receptor (IFN-R) and is characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

The ability to neutralize the biological properties of type I-IFN can be estimated as a function of the capacity of the monoclonal antibody to neutralize the antiviral activity of the type I-IFN. Such a test is relevant in order to determine whether the antibody assayed is included within the scope of the invention, although it is clear that the biological properties of type I-IFN are not limited to its antiviral properties. Detailed procedures are given in the examples in order to enable to perform such a test of the antiviral activity. The cells tested can advantageously be Daudi-cells, which affinity for the type I-IFN is well known. The main steps of such a test would consist in :

- incubating a determined concentration of human cells responsive to human type I-IFN, with human type I-IFN in the presence of a determined concentration of monoclonal antibodies to be assayed, for a time sufficient to allow the formation of a complex between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the incubated cells with a determined virus, in a determined concentration,
- washing the cells,
- resuspending the cells in culture medium,
- incubating for a time sufficient to allow virus replication ;
- lysing the cells ;
- measuring the virus replication, or measuring the inhibition of the cytopathic effect.

The ability of the monoclonal antibodies of the invention to neutralize the biological properties of the human type I-IFN can be modulated as a function of the dose of antibodies used. Accordingly a 100% inhibition of the biological properties, or a partial inhibition can be obtained.

According to another embodiment of the present invention, the monoclonal antibodies directed against the human type I-IFN receptor, are further characterized by the fact that they are capable of inhibiting the binding of a human type I-IFN, to the human IFN-R.

A monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN to its receptor, can be selected by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies or a hybridoma culture supernatant containing monoclonal antibodies to be assayed, with human cells capable of harboring IFN-R ;
- adding labelled human type I-IFN, in a determined concentration, to the above preincubated medium ;

- incubating the medium containing the human cells, the monoclonal antibodies and the labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the labelled type I-IFN by counting the amount of attached labelled type I-IFN.

Some of the monoclonal antibodies of the invention, have also the capacity to neutralize the antiproliferative properties of the human type I-IFN. This property can also be assayed on Daudi cells, by performing the following steps :

- allowing cells to grow in presence of human type IFN and determined concentration of mAb ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the human type I-IFN.

One property of a monoclonal antibody according to the invention resides in its capacity to recognize the extracellular domain of the human IFN receptor. This property of the monoclonal antibody can be assayed on human cells bearing the natural human receptor but also on the extracellular domain of a recombinant IFN-R such as expressed in a procaryotic cell, for instance in E.coli or a recombinant IFN-R such as expressed in a eucaryotic cell such as mamalian cell for instance a CHO-cell.

This receptor can indeed present different properties, depending on the fact that it is produced in a procaryotic or eucaryotic cell and accordingly

depending on the fact that the post-translational maturation occurred or not. The inventors interestingly showed that relevant assays, to evaluate the capacity of a monoclonal antibody according to the invention i.e. to recognize the cellular IFN-R, can be performed on a recombinant receptor expressed in mamalian cells. As a matter of fact, such recombinant receptor has the same properties as the cellular receptor, as far as its recognizing activity is concerned.

Monoclonal antibodies of the invention can be obtained against various forms of the receptor, including the complete receptor, a particular domain or a peptide characteristic of the aminoacid sequence of the receptor represented in figure 3.

Monoclonal antibodies of the invention can for example be prepared against the soluble form of the receptor. A hydrosoluble polypeptide corresponding to the soluble form of the INF-R is described on figure 2. According to the present invention, a soluble form of the IFN-R corresponds to a peptide or a polypeptide, capable of circulating in the body.

Other monoclonal antibodies according to the invention can also be prepared against a peptide comprised in the extracellular domain of the receptor as described on figure 2. An advantageous peptide corresponds for instance to the aminoacid sequence comprised between aminoacid 1 and aminoacid 229. According to another embodiment of the invention, the antibodies can be prepared against a polypeptide modified by substitution of one or more amino acids, provided that antibodies directed against the non modified extracellular domain of the IFN-R, recognize the modified polypeptide or peptide.

Preferred monoclonal antibodies according to the invention are those which are of the IgG1 type.

Among the antibodies of the invention, an antibody which has the capacity of inhibiting the binding of the type I-IFN to its receptor is preferably characterized in that it inhibits the in vitro binding of human type IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0.5 to 2 $\mu\text{g/ml}$.

The inventors have shown that the high affinity binding capacity of a monoclonal antibody is not sufficient to ensure that this antibody will be able to inhibit the binding activity of the human type I-IFN to the IFN-R. Nevertheless the high affinity binding capacity of the monoclonal antibody is necessary to investigate further the ability of the antibody to inhibit the binding of the type I-IFN to its cellular receptor.

Another monoclonal antibody is characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

According to another embodiment a monoclonal antibody is also characterized in that it neutralizes in vitro the antiproliferative activity of human type IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

A particular group of monoclonal antibodies according to the invention is characterized in that it

neutralizes the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 50 $\mu\text{g/ml}$, preferably 1 to 20 $\mu\text{g/ml}$, for a concentration of type I-IFN in the range of 1 to 1000 units with reference to the international standard MRC 69/19.

Advantageously, the monoclonal antibody according to the invention is such that these antibodies do not bind to the human receptor for IFN gamma.

One particular antibody satisfying the requirements of the invention, is such as it directed against an epitope on the amino-acid sequence comprised between amino-acid 27 and amino-acid 427 of the extracellular domain of the human IFN-R as represented on figure 2.

One particularly interesting monoclonal antibody according to the invention is the antibody designated 64G12 under n° 92022605 which has been deposited at the ECACC (European Collection of Animal Cell Cultures Porton Down Salisbury, Wiltshire SP4 056, United Kingdom) on February 26, 1992.

These antibodies may be prepared by conventional methods involving the preparation of hybridoma cells by the fusion of myeloma cells and spleen cells of an animal immunized beforehand with the peptide antigen, on the conditions such that the antigen against which the antibodies are formed is constituted by the extracellular domain of IFN-R or any polypeptide or peptide of this domain.

The hybridomas are constructed according to the protocols of Kohler and Milstein (Nature, 1974, 256: 495-497). For example the hybridomas are derived from

the fusion of the spleen cells above described with NS1 mouse (BalbC) HGPRT⁺ as myeloma cell.

A second procedure for the production of monoclonal antibodies according to the invention, consists in carrying out the fusion between B-cells of blood immortalized with the Epstein/Barr virus and human B lymphocytes placed beforehand in contact with the extracellular domain or a fragment thereof of the IFN-R, against which it is decided to form monoclonal antibodies. B-cells placed in contact beforehand with the extracellular domain of IFN-R or fragment thereof against which it is decided to form monoclonal antibodies, may be obtained by in vitro culture contacted with the antigens, the recovery of the B-cells coated with these antigens being preceded by one or several cycles of stimulation.

The invention thus concerns human antibodies as obtained by carrying out the above procedure, having the above defined properties.

The invention also aims at providing a monoclonal antibody characterized in that the variable or complementary determining regions of its heavy and/or light chains are grafted on the framework and/or constant regions of a human antibody.

The invention further provides a composition having antagonist properties for the biological properties of the human type I-IFN, characterized in that it comprises monoclonal antibodies as defined above.

Accordingly the invention provides a pharmaceutical composition characterized in that it comprises monoclonal antibodies as defined above, together with an appropriate pharmaceutical vehicle.

The invention also concerns the use of a monoclonal antibody as defined above, for the manufacture of a drug for the treatment or prophylaxis of a pathological state or symptoms associated with overproduction of type-I-IFN.

According to a first example, the antibodies can be used in a pharmaceutical composition, for the treatment of allograft rejection.

According to another example, antibodies of the invention are used as active principle in a pharmaceutical composition for the treatment of autoimmune and inflammatory diseases. Such diseases include systemic lupus erythematosus, type 1 diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, acquired immunodeficiency syndrome (AIDS), and severe combined immunodeficiency disease.

Treatment of acute virus diseases can also be performed with the antibodies of the invention. As example upper respiratory tract infections, chronic virus infections such as those due to measles virus, can be performed.

The antibodies of the invention can also be used for the in vitro diagnosis of the presence of the human type I-IFN receptor or cells.

Further details and additional information will arise from the description from the description of the examples and from the figures.

FIGURES

- Figure 1 : binding of ^{125}I -labelled monoclonal antibodies 34F10 and 64G12 to :

- A : Daudi cells
- B : Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was measured by incubation with a 100 fold excess of cold antibodies and subtracted from total counts.

- Figure 2 : nucleotide and corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthesized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in figure 2.

- Figure 3 : nucleotide and corresponding amino-acid sequence of the human IFN-R.

E X A M P L E S**EXAMPLE 1 :**Synthesis of the soluble receptors
Synthesis in E.coli

A fragment of DNA containing the sequence coding for the extracellular domain (amino acids 27 to 427) of the human INF-R (figure 2), in which an extra-sequence coding for 5 histidyl residues was introduced just before the termination codon, was cloned in the expression vectors pKK233-2. This fragment was produced by the Polymerase Chain Reaction (PCR) and the resulting plasmids were sequenced to confirm both in-frame insertion with the Shine-Dalgarno sequence and the appropriate sequence coding for the receptor.

The poly-histidyl tail introduced into the recombinant protein enables it to be purified rapidly by affinity chromatography on a chelated nickel support (NTA column) as described previously (Hochuli E. et al, Bio/technology, 1988, 1321-1325).

The plasmid was introduced into the E.coli strain, JM105, and protein synthesis induced by addition of IPTG to the culture medium (pKK233-2, tac promoter).

Proteins were extracted from the bacterial pellet and the soluble receptor purified to homogeneity by affinity chromatography as described hereafter. This procedure yielded a protein that migrates as 2 bands around 50 kDa under reducing conditions and three bands under non-reducing conditions. The maximum concentration of the protein obtained by different procedures was approximately 20µg/ml.

The N-terminal sequence of the two proteins detected by gel electrophoresis has shown that both proteins are the expected fragment of the receptor.

Synthesis and purification of an unglycosylated soluble receptor :

Bacterial culture (250ml)

|

IPTG induction 3h

|

cell pellet

6M Guanidine hydrochloride pH8

|

centrifugation

|

NTA column:

Washes pH 8 urea 8M

pH 6,3 urea 8M

pH 5.9 urea 8M

|

Elution pH 4 urea 8M

|

refolding dilution, dialysis

|

against Tris 0,1 M pH9

dialysis PBS

Using the same PCR approach, we also constructed an expression vector coding for the IFN-R amino acid sequence 1-427, with an additional 5-histidyl residues at the C-terminus, inserted in expression vector pXMT-3. The exact nucleotide sequence of the insert was also confirmed.

The resulting plasmid was introduced by electroporation into Cos7 cells for transient expression and the recombinant protein was purified to homogeneity by affinity chromatography followed by ion exchange chromatography on mono-Q (Pharmacia) as described hereafter.

Purification of the soluble IFN-R from Cos7 cells

preparative electroporation of
cos cells

| 18 h
|

serum free medium
|
|

supernatants taken after 48h, 72h, 96h
|
|

concentration
|
|

NTA column
|
|

Wash PBS

elution 0.1 M NaOAc pH 5.5
|
|

neutralization

concentration, 30 000 cut off
|
|

Mono Q (0-0.5 M Na Cl)

This purification yielded to a 76 kDa protein whose N-terminal sequence corresponds to the predicted receptor sequence with some heterogeneity in the processing of the leader sequence.

EXAMPLE 2 :

Production of monoclonal antibodies against the interferon type I receptor

1) Production of the monoclonal antibodies

Mice were immunized by injection of recombinant soluble interferon (r sIFN-R) purified from E.coli or from a culture supernatant of Cos7 cells. Initially mice were injected both intraperitoneally and subcutaneously with the purified protein in complete Freund's adjuvant. Subsequently mice were injected once a week intraperitoneally with the purified proteins diluted in buffered saline solution. Ten micrograms of recombinant proteins were injected each time.

After the fourth injection, blood was collected and the presence of specific serum antibodies were tested by both ELISA and Western blot against the recombinant receptor. The strongest responders were then boosted with a total of 10 μ g of antigen half of which was injected intravenously and half intraperitoneally.

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT⁻ myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35:1-32, 1980). Briefly, 5×10^7 spleen cells were fused to 3×10^7 myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as 20×10^7 spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method :

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed SIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.05% Tween 20,

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% Tween 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

3) Identification of reactivity to the natural interferon type I receptor

The reactivity of the monoclonal antibodies (mAbs) recognizing the recombinant sIFN-R was tested against the natural class I receptor expressed at the surface of Daudi cells, by membrane immunofluorescence. Briefly, 5×10^5 Daudi cells were incubated in 100 μ l of culture supernatant of chosen hybridomas for 30 min at 4°C. The cells were then washed 4 times in RPMI medium containing 1% BSA and further incubated with a diluted FITC labelled goat anti-mouse F(ab')₂ for 30 min at 4°C. The cells were finally analyzed by flow cytometry after washing. One of the 35 tested antibodies produced against the E.coli recombinant receptor and 5 of the 6 tested antibodies produced against the COS recombinant receptor were found to recognize the natural receptor on the Daudi cells.

Cloning of these hybridomas was then performed by limiting dilution. The isotype of these mAbs was determined by an ELISA method using isotype specific antibodies. All 6 mAbs were found to be IgG1 with kappa light chains. A summary of the reactivity of these 6 mAbs is given in Table 1.

Monoclonal antibodies were purified from culture supernatants by protein G chromatography.

Tabl 1 :Reactivity of the anti IFN-R monoclonal antibodies

	Reactivity against the recombinant receptor				Reactivity against * the cellular receptor
	E.COLI		COS		immunofluorescence
	ELISA	Western	ELISA	Western	
34F10	+	+	+	+	+
64G12	+	+	+	+	+
63F6 64G2 64D10 65D8	-	-	+	+ weak	+

* measured on Daudi cells

EXAMPLE 3 :Inhibition of the binding of interferon to human cell lines

Inhibition of interferon binding to human cells was assayed as follows. 10^6 cells were preincubated at 4°C for 30 min with various dilutions of hybridoma culture supernatants or purified mAbs or with medium alone. 125 I-labelled IFN alpha 8 or alpha 2 was added at the concentration of 100pM and cells incubated for a further 2 hours at 4°C. These incubations were performed in RPMI medium containing 20mM HEPES pH 7.4 and 10% foetal calf serum (FCS). The cells were finally washed 4 times with RPMI - 1% FCS and counted to determine bound radioactivity.

The mAb secreted by the hybridoma line 64G12 (later named mAb 64G12) was shown in this assay to inhibit the binding of labelled IFN to the cells in a dose-dependent manner. 50% inhibition of binding to the Daudi cells (Burkitt lymphoma cell line ; Klein et al., Cancer Research, 28:1300-1310, 1968) was obtained at a mAb concentration of 0.4 µg/ml. The same inhibition was obtained with K562 cells (chronic myelogenous leukemia, Lozzio and Lozzio, Cell, 45:321-334, 1975) but 50% inhibition was obtained at 11 µg/ml for HL60 cells (Promyelocytic leukemia, Collins S.J. et al., Nature, 270:347-349, 1977) and 60 µg/ml for Ly28 cells (Klein G. et al. Int. J. Cancer, 10:44-57, 1972).

Table 2 :

The inhibition of binding of labelled IFN alpha 2 to various cell lines by mAb64G12

Cell lines	Concentration of mAb which gives 50% inhibition of binding
Daudi K562	0,4 µg/ml
HL60	11 µg/ml
Ly28	60 µg/ml

The difference in the mAb concentration at which 50% inhibition of binding of IFN is obtained has been investigated by direct binding of ¹²⁵I-labelled mAbs 64G12 and 34F10 to the same cell lines and Scatchard

plot analysis of the results. In the concentration range of 0.1 to 1.5 $\mu\text{g/ml}$, a high affinity binding of the mAb 34F10 ($\approx 10\text{nM}$) was seen on all cell lines whereas a high affinity binding of mAb 64G12 was only detected on Daudi and K562 cells (Figure 1).

EXAMPLE 4 :

Inhibition of the function of type I interferon

Functional inhibition of type I interferon by the purified mAb 64G12 was demonstrated in an antiviral assay on Daudi cells using either recombinant IFN alpha 2, IFN beta and IFN omega, or purified Namalwa and leucocyte interferons, and in an antiproliferative assay with recombinant IFN alpha 2.

* Antiviral activity

An antiviral assay on Daudi cells was performed as described (M. Dron and M.G. Tovey, J. Gen. Virol. 64:2641-2647, 1983). Cells ($0.5 \times 10^6/\text{ml}$) were incubated for 24 hours in the presence of interferon and antibodies. 10^6 cells in 1 ml were then infected for 1 hour at 37°C with Vesicular stomatitis virus (VSV) then washed 3 times, resuspended in culture medium and incubated for 18 hours at 37°C . Cells were then lysed by freeze-thawing and virus replication measured by titration of the supernatants on L929 cells. A dose-dependent inhibition of the antiviral activity of the various subtypes of type I IFN was demonstrated for the purified mAb 64G12.

For the antiviral assay with the Wish cells, cells were incubated for 24 hours with various concentrations of interferons in the presence of the mAbs prior to challenge with VSV. In this assay, the mAb 64G12 was demonstrated to block completely the antiviral activity of Leukocyte IFN (50U/ml), recombinant IFN alpha 2 (50U/ml) and interferon from the sera of AIDS patients (50, 75 and 150U/ml).

* antiproliferative activity

For the antiproliferative assay, Daudi cells were seeded at a concentration of 10^5 cells per ml in a 96 well plate in the presence of interferon and purified inhibitory or control antibody. Cells were then counted after 24, 48 and 72 hours with a Coulter counter and checked for viability by trypan blue exclusion. Purified mAb 64G12 demonstrated a dose-dependent inhibition of the antiproliferative activity of interferon alpha 2.

C L A I M S

1. Monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

2. Monoclonal antibody directed against the human type I IFN-R according to claim 1, characterized by its capacity to inhibit the binding of a human pathological type I-IFN, to the IFN-R.

3. Monoclonal antibody according to claim 1 or 2, which is obtainable from a hybridoma cell prepared by fusion of a myeloma cell with spleen cells from an animal previously immunized with the soluble form of the human IFN-R.

4. Monoclonal antibody according to anyone of claims 1, 2 or 3, characterized in that it recognizes an epitope on a soluble form of the human cellular IFN-R or of a recombinant IFN-R.

5. Monoclonal antibody according to anyone of claims 1 to 4, characterized in that it inhibits in vitro the binding of human type I-IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0,5 to 2 $\mu\text{g/ml}$.

6. Monoclonal antibody according to anyone of claims 1 to 5, characterized in that it neutralizes in vitro the antiproliferative activity of the human type I-IFN, on cells highly responsive to this human type I-IFN,

for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

7. Monoclonal antibody according to anyone of claims 1 to 6, characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells poorly responsive to this human type I-IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

8. Monoclonal antibody according to anyone of claims 1 to 7, characterized in that it does not bind to the human receptor of the IFN gamma.

9. Monoclonal antibody according to anyone of claims 1 to 8, characterized in that it recognizes an epitope on the aminoacid sequence 27 to 427 of the human IFN-R.

10. Monoclonal antibody according to anyone of claims 1 to 9, characterized in that it neutralizes in vitro the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

11. Monoclonal antibody according to anyone of claims 1 to 10, characterized in that it neutralizes in vitro the antiviral activity of the human class I-IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

12. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is the 64G12 antibody, deposited at the ECACC on February 26, 1992 under n° 92022605.

13. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a humanized antibody, for instance characterized in that the variable or complementary determining regions of its

heavy and light chains are grafted on the framework and constant regions of a human antibody.

14. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a human antibody.

15. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is an IgG1 type antibody.

16. Hybridoma cell, characterized in that it produces monoclonal antibodies according to claims 1 to 13.

17. Composition having antagonist properties to the type I-IFN, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 16.

18. Pharmaceutical composition, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 17, together with an appropriate pharmaceutical vehicle.

19. Use of a monoclonal antibody according to anyone of claims 1 to 17, for the manufacture of a drug for the treatment or prophylaxis of a pathological state associated with proliferative cell activity and/or viral cell infection.

20. Process for the selection of a monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN, to the IFN-R, characterized by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies according to anyone of claims 1 to 15 or a hybridoma culture supernatant containing monoclonal antibodies, with human cells susceptible of harboring IFN-R ;
- adding labelled human type I-IFN in a determined concentration, to the above preincubating medium ;

- incubating the medium containing the human cells, monoclonal antibodies and labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the type I-IFN, by counting the amount of attached labelled type I-IFN.

21. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiproliferative activities of the type I-IFN, on human cells characterized by the steps of :

- allowing cells to grow in the presence of human type I-IFN and in the presence of a determined concentration of monoclonal antibodies according to anyone of claims 1 to 15 ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the type I-IFN.

22. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiviral activities of the natural, non pathological or pathological type I-IFN on human cells, characterized by the steps of :

- incubating cells with type I-IFN and monoclonal antibodies according to anyone of claims 1 to 15, in determined concentrations, for a time sufficient to allow the formation of a complex

between the monoclonal antibodies and the IFN-R of the human cells and/or between the typ I-IFN and the IFN-R of the human cells ;

- infecting the above incubated cells with a determined concentration of a virus ;
- washing the cells ;
- resuspending the cells in culture medium ;
- incubating for a time sufficient to allow the replication of the virus ;
- lysing the cells and ;
- measuring the virus replication or measuring the inhibition of the cytopathic effect.

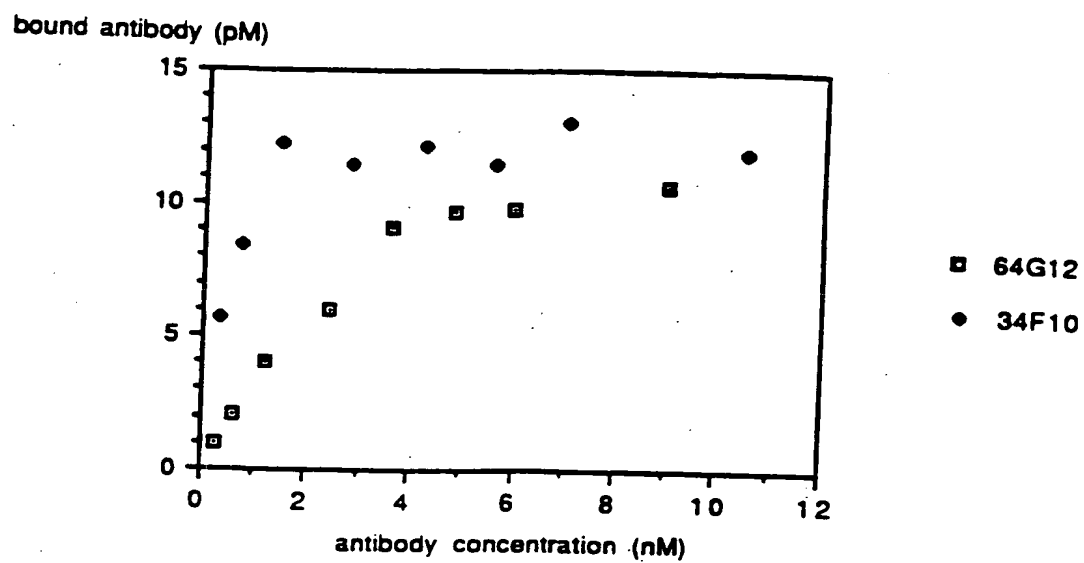
A B R I D G M E N TMONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The invention relates to a monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

It further concerns their use for the diagnosis.

A



B

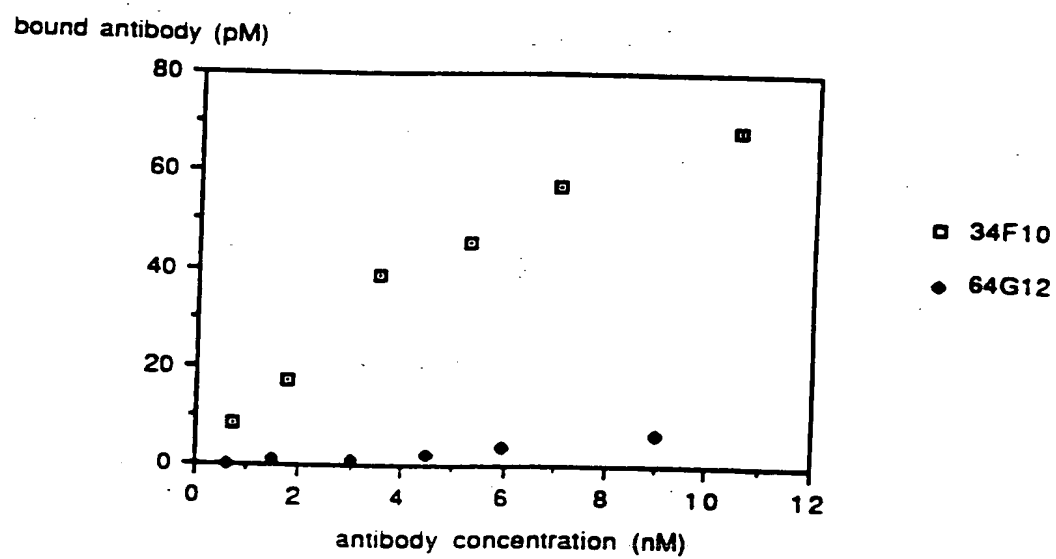


FIGURE 1

CTGCAGGGATCTGCGCGGGCTCCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 2A

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn MET Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu

AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr MET Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA TGAGGTACC
Asn Thr Ser Lys

1334

FIGURE 2B

CTGCAGGGATCTCGGGCGGCTCCAC

ATG ATG GTC GTC CTC CTG GGC GCG ACC ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 3A

ATT GAA AAT ATA AGC ACA ATT GCT ACA GTA GAA GAA ACT AAT CAA ACT GAT GAA
 Ile Glu Asn Ile Ser Thr Ile Ala Thr Val Glu Glu Thr Asn Gln Thr Asp Glu

GAT CAT AAA AAA TAC AGT TCC CAA ACT AGC CAA GAT TCA GGA AAT TAT TCT AAT
 Asp His Lys Lys Tyr Ser Ser Gln Thr Ser Gln Asp Ser Gly Asn Tyr Ser Asn

GAA GAT GAA AGC GAA AGT AAA ACA AGT GAA GAA CTA CAG CAG GAC TTT GTA TGA
 Glu Asp Glu Ser Glu Ser Lys Thr Ser Glu Glu Leu Gln Gln Asp Phe Val

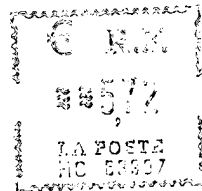
CCAGAAATGAACTGTGTCAAGTATAAGGTTTTTCAGCAGGAGTTACACTGGTACC

↑
1697

FIGURE 3C



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25 euros
B1608ACIA

NON RECLAMÉ
RETOUR A L'ENVOYEUR

LETTRE

pas

→ VMAMNH RECOMMANDE

REÇU LE

25 SEP. 2001

E. GUTMANN - Y. PLASSERAUD S.A.

LA POSTE

AVIS DE RÉCEPTION
DE VOTRE ENVOI
RECOMMANDE

RA 6156 2266 3FR
05/09/01

Présenté le : 05/09/01

Distribué le :

Signature du destinataire :

NON RECLAMÉ
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75008 Paris

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30 bis, rue du Cardinal Lemoine
75231 PARIS CEDEX 05

756 000 000 RCS NANTERRE
RCS NANT.

SIREN 366 000 000 RCS NANTERRE

AVIS DE RÉCEPTION

ANNEX 21

3rd sending of the same package to Ms. MAGUIRE on October 5, 2001. This package was returned to us on October 29, 2001, as being yet again not collected by the addressee.



ERNEST GUTMANN - YVES PLASSERAUD S.A.
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Par RECOMMANDÉ A/R

Madame Deborah MAGUIRE
24 rue Maître-Albert

75005 PARIS

3° envoi

BDS
17283/23

VOTRE REFERENCE :

NOTRE REFERENCE :

LE

B1608ACA – JAZ/VMA/MNH

05 octobre 2001

Objet : Brevet américain n° US 5,919,453 du 6 juillet 1999
Demande divisionnaire n° 09/240,675 déposée le 2 février 1999
au nom de MEDISUP INTERNATIONAL N.V.
Inventeurs : BENOIT, MEYER, MAGUIRE, PLAVEC, TOVEY

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Florence LAZARD, cpi
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Véronique MARCADÉ
Denis BOURGAREL
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°US patent attorney
°conseil européen en marques
OHM/OHIM

(1) Agence de Lyon
(2) Agence d'Alicante

Chère Madame,


En tant qu'inventeur désigné dans le brevet américain cité en référence, vous avez signé, le 11 novembre 1994, une déclaration donnant pouvoir à nos correspondants de la société Foley & Lardner, afin qu'ils vous représentent auprès de l'Office Américain des Brevets. Une copie de cette déclaration est ci-jointe.

Dans le cadre d'une demande de brevet divisionnaire, portant sur des peptides particuliers reconnus par l'anticorps monoclonal 64G12, l'Examineur américain en charge de ce dossier considère que cette déclaration est défectueuse car certaines corrections manuscrites y ont été apportées sans être datées et paraphées. Il nous demande donc de lui fournir une nouvelle déclaration.

Vous trouverez donc ci-joint, une copie du texte initialement déposé ainsi qu'une copie des revendications de la demande divisionnaire. Vous trouverez aussi un exemplaire d'une déclaration supplémentaire, que nous vous prions de bien vouloir nous renvoyer après l'avoir datée et signée. Nous attirons votre attention sur le contenu du sixième paragraphe de cette déclaration, qui précise que vous avez lu et compris la description et l'ensemble des revendications ci-jointes. Si vous avez la moindre question à ce sujet, n'hésitez pas à nous contacter. Par ailleurs, si vous

décidez de ne pas signer cette déclaration, nous vous remercions de bien vouloir nous en avvertir.

Veuillez agréer, Chère Madame, l'expression de nos meilleures salutations.



Véronique MARCADÉ



Julia ANDRAL-ZIURYS

- P.J. : - Déclaration du 11/11/94
- Texte initialement déposé et revendications de la demande divisionnaire
- Déclaration supplémentaire

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST TYPE 1 INTERFERON

the specification of which (check one)

☐ is attached hereto

☒ was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by a amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before the of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
92400902.0	European	31/March/1992	Yes

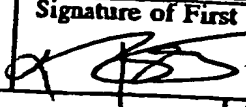
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; John J. Feldhaus, Reg. No. 28,822; Donald D. Jeffery, Reg. No. 19,980; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Neil Meloy, Reg. No. 22,749; George E. Quinn, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 26,665; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115.

Send all correspondence to **FOLEY & LARDNER**, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696.
Address telephone communications to Bernhard D. Saxe at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Post Office Address 24, rue Jonquoy, F-75014 Paris, France			

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Residence Address Paris, France	Country of Citizenship FRANCE LUXEMBOURG	
Post Office Address 14, square Adanson, F-75005 Paris, France		

Deborah

Full Name of Third Inventor Deborah MAGUIRE	Signature of Third Inventor <i>[Signature]</i>	Date 11/11/94
Residence Address Paris, France	Country of Citizenship FRANCE AUSTRALIA	
Post Office Address 24, rue Maitre-Albert, F-75005 Paris, France		

Full Name of Fourth Inventor Ivan PLAVEC	Signature of Fourth Inventor <i>[Signature]</i>	Date 11/11/94
Residence Address Fresnes, France	Country of Citizenship FRANCE CROATIA	
Post Office Address 1, allée du Capitaine-Dupont F-92260 Fresnes, France		

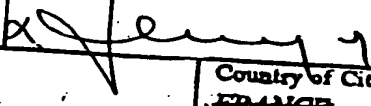
Full Name of Fifth Inventor Michael G. TOVEY	Signature of Fifth Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
Residence Address Paris, France	Country of Citizenship GREAT BRITAIN	
Post Office Address 6, rue des Quatrefoies, F-75005 Paris, France		


Full Name of Second Inventor Francois MEYER	Signature of Second Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 14, square Adanson, F-75005 Paris, France		

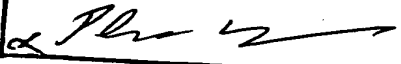
Full Name of Third Inventor Deborah MAGUIRE	Signature of Third Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
Residence Address Paris, France	Country of Citizenship FRANCE	
Post Office Address 24, rue Maitre-Albert, F-75005 Paris, France		

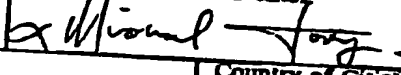
Full Name of Fourth Inventor Ivan PLAVEC	Signature of Fourth Inventor <i>[Signature]</i>	Date <i>[Blank]</i>
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MONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The interferons (IFN) constitute a group of secreted proteins which exhibit a wide range of biological activities and are characterized by their capacity to induce an antiviral state in vertebrate cells (I. Gresser and M.G. Tovey Biochem Biophys. Acta 516:231, 1978). There are three antigenic classes of IFN : alpha (α), beta (β) and gamma. IFN α and IFN β together are known as the type I interferon.

Natural type I human interferon comprises 12 or more closely related proteins encoded by distinct genes with a high degree of structural homology (Weissmann and Weber, Prog. Nucl. Acid. Res. Mol. Biol. 33:251, 1986).

The human IFN α locus comprises two subfamilies. The first subfamily consists of 14 non allelic genes and 4 pseudogenes having at least 80% homology. The second subfamily, α II or omega (ω), contains 5 pseudogenes and 1 functional gene which exhibits 70% homology with the IFN α genes (Weissmann and Weber 1986).

The subtypes of IFN α have different specific activities but they possess the same biological spectrum (Streuli et al. PNAS-USA 78:2848, 1981) and have the same cellular receptor (Agnat M. et al. in "Interferon 5" Ed. I. Gresser p. 1-22, Academic Press, London 1983).

The interferon β (IFN β) is encoded by a single gene which has approximately 50% homology with the IFN α genes.

The interferon α subtypes and interferon β bind to the same receptor on the cell surface.

The interferon gamma (IFN gamma) is also encoded by a single copy, which has little homology with the IFN α and IFN β genes. The receptor for IFN gamma is distinct from the receptor of the α and β interferons.

For the purpose of the present invention the receptor of α and β classes of IFN will be designated IFN-R. This represents natural type I receptor. The group of proteins forming natural interferon α will be designated IFN α , and type I-IFN will represent both natural IFN α , IFN ω , and IFN β .

Despite the fact that interferon is a potent antiviral agent, there is considerable evidence to suggest, that many of the characteristic symptoms of acute virus diseases such as upper respiratory tract infections are caused by an overproduction of interferon alpha. Furthermore, IFN alpha has been shown to contribute to the pathogenesis of certain chronic virus infections in experimental animals and the available evidence suggests that this is also the case for certain human chronic virus diseases such as those due to measles virus.

The interferons α are also potent immuno-regulatory molecules which stimulate polyclonal B-cell activation, enhance NK cell cytotoxicity, inhibit T-cell functions, and modulate the expression of the major histocompatibility complex (MHC) class 1 antigens, all of which are implicated in the induction of autoimmunity and in graft rejection. The abnormal production of interferon α is associated with a number of autoimmune diseases and inflammatory disorders including systemic lupus erythematosus (SLE), type I diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, the acquired immunodeficiency syndrome (AIDS) and severe

combined immunodeficiency disease. The presence of interferon α in the serum of patients with systemic lupus is correlated with both the clinical and humoral signs of increased disease activity. The production of interferon α in HIV positive subjects is also highly predictive of disease evolution.

Administration of interferon α has been reported to exacerbate underlying disease in patients with psoriasis and multiple sclerosis and to induce a SLE like syndrome in patients without a previous history of autoimmune disease. Interferon α has also been shown to induce glomerulonephritis in normal mice and to accelerate the outset of the spontaneous autoimmune disease of NZB/W mice.

Interferon α is also produced during the course of graft-versus-host disease (GVHD) in parallel with the enhanced NK cell activity characteristic of systemic GVHD. Interferon α is the principal modulator of NK cell cytotoxicity and administration of interferon α has been shown to enhance the intestinal consequences of GVHD in normal mice.

The object of the present invention is to provide new antagonists against the biological activities of the human type I-IFN. These antagonists could be used for therapeutical, including prophylaxis purposes, in cases where the type I-IFN (IFN α/β) is abnormally produced and when this abnormal production is associated with pathological symptoms. Such antagonists could also be used for the diagnosis of various diseases or for the study of the evolution of such diseases.

In order to define such antagonists, the inventors have taken into account the fact that the human natural type I-IFN is in fact constituted of a mixture of

interferons (subspecies) and the fact that the composition of this association of different subtypes of interferons varies both quantitatively and qualitatively.

Some natural interferons, such as the ones secreted by Namalwa cells (Namalwa interferon) or leukocyte (leucocyte interferon) have been studied in detail (N.B. Finter and K.H. Fautes, Interferon 2, 1980, p. 65-79 I. Gresser Editor Academic Press ; K. Cantell et al, Interferon 1, 1979 p. 2-25, I. Gresser Editor Academic Press) and were used by the inventors to define natural type I interferons.

In some pathological cases, like AIDS, interferons having some special properties have been described (O.T. Preble et al, Annals of New-York Academy of Sciences p. 65-75). This interferon involved in pathological cases like AIDS nevertheless binds to the same receptor, as described above.

One object of the present invention is to provide an antagonist of the type I-IFN, which would be able to inhibit or neutralize, to a determined extent, the biological properties of the human type I-IFN, that is to say, to neutralize in vivo a mixture of α , β , ω subspecies.

Accordingly the inventors have defined antibodies, especially monoclonal antibodies, which have the property of being antagonists to the type I-IFN. These antibodies are directed against the human type I-IFN receptor.

The invention thus also concerns the use of the monoclonal antibodies for the preparation of pharmaceutical compositions, useful for the treatment of symptoms associated with the abnormal production of

type I-IFN. These monoclonal antibodies are also appropriate for the preparation of diagnosis reagents.

A monoclonal antibody according to the present invention is directed against the human type I-interferon receptor (IFN-R) and is characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

The ability to neutralize the biological properties of type I-IFN can be estimated as a function of the capacity of the monoclonal antibody to neutralize the antiviral activity of the type I-IFN. Such a test is relevant in order to determine whether the antibody assayed is included within the scope of the invention, although it is clear that the biological properties of type I-IFN are not limited to its antiviral properties. Detailed procedures are given in the examples in order to enable to perform such a test of the antiviral activity. The cells tested can advantageously be Daudi-cells, which affinity for the type I-IFN is well known. The main steps of such a test would consist in :

- incubating a determined concentration of human cells responsive to human type I-IFN, with human type I-IFN in the presence of a determined concentration of monoclonal antibodies to be assayed, for a time sufficient to allow the formation of a complex between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the incubated cells with a determined virus, in a determined concentration,
- washing the cells,
- resuspending the cells in culture medium,
- incubating for a time sufficient to allow virus replication ;
- lysing the cells ;
- measuring the virus replication, or measuring the inhibition of the cytopathic effect.

The ability of the monoclonal antibodies of the invention to neutralize the biological properties of the human type I-IFN can be modulated as a function of the dose of antibodies used. Accordingly a 100% inhibition of the biological properties, or a partial inhibition can be obtained.

According to another embodiment of the present invention, the monoclonal antibodies directed against the human type I-IFN receptor, are further characterized by the fact that they are capable of inhibiting the binding of a human type I-IFN, to the human IFN-R.

A monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN to its receptor, can be selected by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies or a hybridoma culture supernatant containing monoclonal antibodies to be assayed, with human cells capable of harboring IFN-R ;
- adding labelled human type I-IFN, in a determined concentration, to the above preincubated medium ;

- incubating the medium containing the human cells, the monoclonal antibodies and the labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the labelled type I-IFN by counting the amount of attached labelled type I-IFN.

Some of the monoclonal antibodies of the invention, have also the capacity to neutralize the antiproliferative properties of the human type I-IFN. This property can also be assayed on Daudi cells, by performing the following steps :

- allowing cells to grow in presence of human type IFN and determined concentration of mAb ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the human type I-IFN.

One property of a monoclonal antibody according to the invention resides in its capacity to recognize the extracellular domain of the human IFN receptor. This property of the monoclonal antibody can be assayed on human cells bearing the natural human receptor but also on the extracellular domain of a recombinant IFN-R such as expressed in a procaryotic cell, for instance in E.coli or a recombinant IFN-R such as expressed in a eucaryotic cell such as mamalian cell for instance a CHO-cell.

This receptor can indeed present different properties, depending on the fact that it is produced in a procaryotic or eucaryotic cell and accordingly

depending on the fact that the post-translational maturation occurred or not. The inventors interestingly showed that relevant assays, to evaluate the capacity of a monoclonal antibody according to the invention i.e. to recognize the cellular IFN-R, can be performed on a recombinant receptor expressed in mammalian cells. As a matter of fact, such recombinant receptor has the same properties as the cellular receptor, as far as its recognizing activity is concerned.

Monoclonal antibodies of the invention can be obtained against various forms of the receptor, including the complete receptor, a particular domain or a peptide characteristic of the aminoacid sequence of the receptor represented in figure 3.

Monoclonal antibodies of the invention can for example be prepared against the soluble form of the receptor. A hydrosoluble polypeptide corresponding to the soluble form of the INF-R is described on figure 2. According to the present invention, a soluble form of the IFN-R corresponds to a peptide or a polypeptide, capable of circulating in the body.

Other monoclonal antibodies according to the invention can also be prepared against a peptide comprised in the extracellular domain of the receptor as described on figure 2. An advantageous peptide corresponds for instance to the aminoacid sequence comprised between aminoacid 1 and aminoacid 229. According to another embodiment of the invention, the antibodies can be prepared against a polypeptide modified by substitution of one or more amino acids, provided that antibodies directed against the non modified extracellular domain of the IFN-R, recognize the modified polypeptide or peptide.

Preferred monoclonal antibodies according to the invention are those which are of the IgG1 type.

Among the antibodies of the invention, an antibody which has the capacity of inhibiting the binding of the type I-IFN to its receptor is preferably characterized in that it inhibits the in vitro binding of human type IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0.5 to 2 $\mu\text{g/ml}$.

The inventors have shown that the high affinity binding capacity of a monoclonal antibody is not sufficient to ensure that this antibody will be able to inhibit the binding activity of the human type I-IFN to the IFN-R. Nevertheless the high affinity binding capacity of the monoclonal antibody is necessary to investigate further the ability of the antibody to inhibit the binding of the type I-IFN to its cellular receptor.

Another monoclonal antibody is characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

According to another embodiment a monoclonal antibody is also characterized in that it neutralizes in vitro the antiproliferative activity of human type IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

A particular group of monoclonal antibodies according to the invention is characterized in that it

neutralizes the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 50 $\mu\text{g/ml}$, preferably 1 to 20 $\mu\text{g/ml}$, for a concentration of type I-IFN in the range of 1 to 1000 units with reference to the international standard MRC 69/19.

Advantageously, the monoclonal antibody according to the invention is such that these antibodies do not bind to the human receptor for IFN gamma.

One particular antibody satisfying the requirements of the invention, is such as it directed against an epitope on the amino-acid sequence comprised between amino-acid 27 and amino-acid 427 of the extracellular domain of the human IFN-R as represented on figure 2.

One particularly interesting monoclonal antibody according to the invention is the antibody designated 64G12 under n° 92022605 which has been deposited at the ECACC (European Collection of Animal Cell Cultures Porton Down Salisbury, Wiltshire SP4 056, United Kingdom) on February 26, 1992.

These antibodies may be prepared by conventional methods involving the preparation of hybridoma cells by the fusion of myeloma cells and spleen cells of an animal immunized beforehand with the peptide antigen, on the conditions such that the antigen against which the antibodies are formed is constituted by the extracellular domain of IFN-R or any polypeptide or peptide of this domain.

The hybridomas are constructed according to the protocols of Kohler and Milstein (Nature, 1974, 256: 495-497). For example the hybridomas are derived from

the fusion of the spleen cells above described with NS1 mouse (BalbC) HGPRT⁻ as myeloma cell.

A second procedure for the production of monoclonal antibodies according to the invention, consists in carrying out the fusion between B-cells of blood immortalized with the Epstein/Barr virus and human B lymphocytes placed beforehand in contact with the extracellular domain or a fragment thereof of the IFN-R, against which it is decided to form monoclonal antibodies. B-cells placed in contact beforehand with the extracellular domain of IFN-R or fragment thereof against which it is decided to form monoclonal antibodies, may be obtained by in vitro culture contacted with the antigens, the recovery of the B-cells coated with these antigens being preceded by one or several cycles of stimulation.

The invention thus concerns human antibodies as obtained by carrying out the above procedure, having the above defined properties.

The invention also aims at providing a monoclonal antibody characterized in that the variable or complementary determining regions of its heavy and/or light chains are grafted on the framework and/or constant regions of a human antibody.

The invention further provides a composition having antagonist properties for the biological properties of the human type I-IFN, characterized in that it comprises monoclonal antibodies as defined above.

Accordingly the invention provides a pharmaceutical composition characterized in that it comprises monoclonal antibodies as defined above, together with an appropriate pharmaceutical vehicle.

Th invention also concerns the use of a monoclonal antibody as defined above, for the manufacture of a drug for the treatment or profilaxis of a pathological state or symptoms associated with overproduction of type-I-IFN.

According to a first example, the antibodies can be used in a pharmaceutical composition, for the treatment of allograft rejection.

According to another example, antibodies of the invention are used as active principle in a pharmaceutical composition for the treatment of autoimmune and inflammatory diseases. Such diseases include systemic lupus erythematosus, type 1 diabetes, psoriasis, rheumatoid arthritis, multiple sclerosis, Behçet's disease, aplastic anemia, acquired immunodeficiency syndrome (AIDS), and severe combined immunodeficiency disease.

Treatment of acute virus diseases can also be performed with the antibodies of the invention. As example upper respiratory tract infections, chronic virus infections such as those due to measles virus, can be performed.

The antibodies of the invention can also be used for the in vitro diagnosis of the presence of the human type I-IFN receptor or cells.

Further details and additional information will arise from the description from the description of the examples and from the figures.

FIGURES

- Figure 1 : binding of ^{125}I -labelled monoclonal antibodies 34F10 and 64G12 to :

- A : Daudi cells

- B : Ly28 cells

Briefly, 10^6 cells were incubated for 2 hours at 4°C in presence of different amounts of the labelled antibodies diluted in RPMI medium containing 10% fetal calf serum (FCS). The cells were then washed 4 times in RPMI-1% FCS and counted for bound radioactivity. Nonspecific binding was measured by incubation with a 100 fold excess of cold antibodies and subtracted from total counts.

- Figure 2 : nucleotide and corresponding amino-acid sequence of the extracellular domain of the human IFN-R

The monoclonal antibodies were produced against recombinant soluble forms of the human interferon alpha-beta receptor (IFN-R) synthesized in either procaryotic cells (E.coli) or a mammalian cell system (Cos cell). These soluble forms were based on the DNA sequence described in figure 2.

- Figure 3 : nucleotide and corresponding amino-acid sequence of the human IFN-R.

E X A M P L E S**EXAMPLE 1 :**Synthesis of the soluble receptorsSynthesis in E.coli

A fragment of DNA containing the sequence coding for the extracellular domain (amino acids 27 to 427) of the human INF-R (figure 2), in which an extra-sequence coding for 5 histidyl residues was introduced just before the termination codon, was cloned in the expression vectors pKK233-2. This fragment was produced by the Polymerase Chain Reaction (PCR) and the resulting plasmids were sequenced to confirm both in-frame insertion with the Shine-Dalgarno sequence and the appropriate sequence coding for the receptor.

The poly-histidyl tail introduced into the recombinant protein enables it to be purified rapidly by affinity chromatography on a chelated nickel support (NTA column) as described previously (Hochuli E. et al, Bio/technology, 1988, 1321-1325).

The plasmid was introduced into the E.coli strain, JM105, and protein synthesis induced by addition of IPTG to the culture medium (pKK233-2, tac promoter).

Proteins were extracted from the bacterial pellet and the soluble receptor purified to homogeneity by affinity chromatography as described hereafter. This procedure yielded a protein that migrates as 2 bands around 50 kDa under reducing conditions and three bands under non-reducing conditions. The maximum concentration of the protein obtained by different procedures was approximately 20µg/ml.

The N-terminal sequence of the two proteins detected by gel electrophoresis has shown that both proteins are the expected fragment of the receptor.

Synthesis and purification of an unglycosylated soluble receptor :

Bacterial culture (250ml)

|
|

IPTG induction 3h

|
|

cell pellet

6M Guanidine hydrochloride pH8

|
|

centrifugation

|
|

NTA column:

Washes pH 8 urea 8M

pH 6,3 urea 8M

pH 5.9 urea 8M

|
|

Elution pH 4 urea 8M

|
|

refolding dilution, dialysis

|
|

against Tris 0,1 M pH9

dialysis PBS

Using the same PCR approach, we also constructed an expression vector coding for the IFN-R amino acid sequence 1-427, with an additional 5-histidyl residues at the C-terminus, inserted in expression vector pXMT-3. The exact nucleotide sequence of the insert was also confirmed.

The resulting plasmid was introduced by electroporation into Cos7 cells for transient expression and the recombinant protein was purified to homogeneity by affinity chromatography followed by ion exchange chromatography on mono-Q (Pharmacia) as described hereafter.

Purification of the soluble IFN-R from Cos7 cells

preparative electroporation of
cos cells

| 18 h
|

serum free medium
|
|

supernatants taken after 48h, 72h, 96h
|
|

concentration
|
|

NTA column
|
|

Wash PBS

elution 0.1 M NaOAc pH 5.5
|
|

neutralization

concentration, 30 000 cut off
|
|

Mono Q (0-0.5 M Na Cl)

This purification yielded to a 76 kDa protein whose N-terminal sequence corresponds to the predicted receptor sequence with some heterogeneity in the processing of the leader sequence.

EXAMPLE 2 :

Production of monoclonal antibodies against the interferon type I receptor

1) Production of the monoclonal antibodies

Mice were immunized by injection of recombinant soluble interferon (r sIFN-R) purified from E.coli or from a culture supernatant of Cos7 cells. Initially mice were injected both intraperitoneally and subcutaneously with the purified protein in complete Freund's adjuvant. Subsequently mice were injected once a week intraperitoneally with the purified proteins diluted in buffered saline solution. Ten micrograms of recombinant proteins were injected each time.

After the fourth injection, blood was collected and the presence of specific serum antibodies were tested by both ELISA and Western blot against the recombinant receptor. The strongest responders were then boosted with a total of 10 μ g of antigen half of which was injected intravenously and half intraperitoneally.

2) Cell fusion

Four days after boosting, spleen cells from the immunized animal were collected and fused to NS1 (mouse) (Balbc) HGPRT⁻ myeloma cells according to the method described by S. Fazekas et al. (J. Immunol. Methods 35:1-32, 1980). Briefly, 5×10^7 spleen cells were fused to 3×10^7 myeloma cells in 1ml of polyethylene glycol solution and distributed in five 96 well plates on a peritoneal macrophage feeder layer in HAT (hypoxanthine, aminoprotein and thymidine) medium. This procedure was repeated 4 times as 20×10^7 spleen cells were obtained from the immunized mouse. Screening for specific hybridomas was undertaken when large colonies were detectable in culture wells.

For the screening, presence of specific antibodies was determined by a direct ELISA method :

a) ELISA plates were coated overnight at 4°C with purified E.coli-expressed or Cos7 cell-expressed sIFN-R diluted in PBS. Plates coated with BSA were used to detect non specific binding,

b) Plates were saturated by incubation with 3% BSA in PBS for 1 hour at 37°C,

c) Plates were incubated for 4 hours at room temperature with hybridoma supernatants diluted 1 in 4 with PBS-0.05% Tween 20,

d) Bound antibodies were detected by a two step procedure, comprising a first incubation with goat anti-mouse biotinylated immunoglobulin followed by streptavidin-horseradish peroxidase complex (both from Amersham and diluted 1/1000 in PBS-0.05% Tween 20).

Positive antibody secreting hybridomas were passaged in 24 well plates on a spleen cell feeder layer and their reactivity was again checked by ELISA, and Western-blot.

3) Identification of reactivity to the natural interferon type I receptor

The reactivity of the monoclonal antibodies (mAbs) recognizing the recombinant sIFN-R was tested against the natural class I receptor expressed at the surface of Daudi cells, by membrane immunofluorescence. Briefly, 5×10^5 Daudi cells were incubated in 100 μ l of culture supernatant of chosen hybridomas for 30 min at 4°C. The cells were then washed 4 times in RPMI medium containing 1% BSA and further incubated with a diluted FITC labelled goat anti-mouse F(ab')₂ for 30 min at 4°C. The cells were finally analyzed by flow cytometry after washing. One of the 35 tested antibodies produced against the E.coli recombinant receptor and 5 of the 6 tested antibodies produced against the COS recombinant receptor were found to recognize the natural receptor on the Daudi cells.

Cloning of these hybridomas was then performed by limiting dilution. The isotype of these mAbs was determined by an ELISA method using isotype specific antibodies. All 6 mAbs were found to be IgG1 with kappa light chains. A summary of the reactivity of these 6 mAbs is given in Table 1.

Monoclonal antibodies were purified from culture supernatants by protein G chromatography.

Tabl 1 :

Reactivity of th anti IFN-R monoclonal antib dies

	Reactivity against the recombinant receptor				Reactivity against * the cellular receptor
	E.COLI		COS		immunofluorescence
	ELISA	Western	ELISA	Western	
34F10	+	+	+	+	+
64G12	+	+	+	+	+
63F6 64G2 64D10 65D8	-	-	+	+	+
				weak	

* measured on Daudi cells

EXAMPLE 3 :Inhibition of the binding of interferon to human cell lines

Inhibition of interferon binding to human cells was assayed as follows. 10^6 cells were preincubated at 4°C for 30 min with various dilutions of hybridoma culture supernatants or purified mAbs or with medium alone. 125 I-labelled IFN alpha 8 or alpha 2 was added at the concentration of 100pM and cells incubated for a further 2 hours at 4°C. These incubations were performed in RPMI medium containing 20mM HEPES pH 7.4 and 10% foetal calf serum (FCS). The cells were finally washed 4 times with RPMI - 1% FCS and counted to determine bound radioactivity.

The mAb secreted by the hybridoma line 64G12 (latter named mAb 64G12) was shown in this assay to inhibit the binding of labelled IFN to the cells in a dose-dependent manner. 50% inhibition of binding to the Daudi cells (Burkitt lymphoma cell line ; Klein et al., Cancer Research, 28:1300-1310, 1968) was obtained at a mAb concentration of 0.4 µg/ml. The same inhibition was obtained with K562 cells (chronic myelogenous leukemia, Lozzio and Lozzio, Cell, 45:321-334, 1975) but 50% inhibition was obtained at 11 µg/ml for HL60 cells (Promyelocytic leukemia, Collins S.J. et al., Nature, 270:347-349, 1977) and 60 µg/ml for Ly28 cells (Klein G. et al. Int. J. Cancer, 10:44-57, 1972).

Table 2 :

The inhibition of binding of labelled IFN alpha 2 to various cell lines by mAb64G12

Cell lines	Concentration of mAb which gives 50% inhibition of binding
Daudi K562	0,4 µg/ml
HL60	11 µg/ml
Ly28	60 µg/ml

The difference in the mAb concentration at which 50% inhibition of binding of IFN is obtained has been investigated by direct binding of ¹²⁵I-labelled mAbs 64G12 and 34F10 to the same cell lines and Scatchard

plot analysis of the results. In the concentration range of 0.1 to 1.5 $\mu\text{g/ml}$, a high affinity binding of the mAb 34F10 ($\approx 10\text{nM}$) was seen on all cell lines whereas a high affinity binding of mAb 64G12 was only detected on Daudi and K562 cells (Figure 1).

EXAMPLE 4 :

Inhibition of the function of type I interferon

Functional inhibition of type I interferon by the purified mAb 64G12 was demonstrated in an antiviral assay on Daudi cells using either recombinant IFN alpha 2, IFN beta and IFN omega, or purified Namalwa and leucocyte interferons, and in an antiproliferative assay with recombinant IFN alpha 2.

* Antiviral activity

An antiviral assay on Daudi cells was performed as described (M. Dron and M.G. Tovey, J. Gen. Virol. 64:2641-2647, 1983). Cells ($0.5 \times 10^6/\text{ml}$) were incubated for 24 hours in the presence of interferon and antibodies. 10^6 cells in 1 ml were then infected for 1 hour at 37°C with Vesicular stomatitis virus (VSV) then washed 3 times, resuspended in culture medium and incubated for 18 hours at 37°C . Cells were then lysed by freeze-thawing and virus replication measured by titration of the supernatants on L929 cells. A dose-dependent inhibition of the antiviral activity of the various subtypes of type I IFN was demonstrated for the purified mAb 64G12.

For the antiviral assay with the Wish cells, cells were incubated for 24 hours with various concentrations of interferons in the presence of the mAbs prior to challenge with VSV. In this assay, the mAb 64G12 was demonstrated to block completely the antiviral activity of Leukocyte IFN (50U/ml), recombinant IFN alpha 2 (50U/ml) and interferon from the sera of AIDS patients (50, 75 and 150U/ml).

* antiproliferative activity

For the antiproliferative assay, Daudi cells were seeded at a concentration of 10^5 cells per ml in a 96 well plate in the presence of interferon and purified inhibitory or control antibody. Cells were then counted after 24, 48 and 72 hours with a Coulter counter and checked for viability by trypan blue exclusion. Purified mAb 64G12 demonstrated a dose-dependent inhibition of the antiproliferative activity of interferon alpha 2.

C L A I M S

1. Monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :
 - it recognizes the extracellular domain of the human IFN-R, and
 - it has a neutralizing capacity against the biological properties of the human type I-IFN.
2. Monoclonal antibody directed against the human type I IFN-R according to claim 1, characterized by its capacity to inhibit the binding of a human pathological type I-IFN, to the IFN-R.
3. Monoclonal antibody according to claim 1 or 2, which is obtainable from a hybridoma cell prepared by fusion of a myeloma cell with spleen cells from an animal previously immunized with the soluble form of the human IFN-R.
4. Monoclonal antibody according to anyone of claims 1, 2 or 3, characterized in that it recognizes an epitope on a soluble form of the human cellular IFN-R or of a recombinant IFN-R.
5. Monoclonal antibody according to anyone of claims 1 to 4, characterized in that it inhibits in vitro the binding of human type I-IFN, to the human cellular IFN-R when it is co-incubated with cells harboring the hu-IFN-R, at a concentration of antibodies equal or inferior to 100 $\mu\text{g/ml}$, preferably equal or inferior to 50 $\mu\text{g/ml}$, advantageously inferior to 20 $\mu\text{g/ml}$, more preferably in the range of approximately 0,5 to 2 $\mu\text{g/ml}$.
6. Monoclonal antibody according to anyone of claims 1 to 5, characterized in that it neutralizes in vitro the antiproliferative activity of the human type I-IFN, on cells highly responsive to this human type I-IFN,

for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

7. Monoclonal antibody according to anyone of claims 1 to 6, characterized in that it neutralizes in vitro the antiproliferative activity of human type I-IFN, on cells poorly responsive to this human type I-IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

8. Monoclonal antibody according to anyone of claims 1 to 7, characterized in that it does not bind to the human receptor of the IFN gamma.

9. Monoclonal antibody according to anyone of claims 1 to 8, characterized in that it recognizes an epitope on the aminoacid sequence 27 to 427 of the human IFN-R.

10. Monoclonal antibody according to anyone of claims 1 to 9, characterized in that it neutralizes in vitro the antiviral activity of the human type I-IFN, on cells highly responsive to this human type I-IFN, for instance Daudi cells at a concentration in a range of 1 to 10 $\mu\text{g/ml}$.

11. Monoclonal antibody according to anyone of claims 1 to 10, characterized in that it neutralizes in vitro the antiviral activity of the human class I-IFN, on cells poorly responsive to this human IFN, for instance Ly28 cells, at a concentration in a range of 50 to 100 $\mu\text{g/ml}$.

12. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is the 64G12 antibody, deposited at the ECACC on February 26, 1992 under n° 92022605.

13. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a humanized antibody, for instance characterized in that the variable or complementary determining regions of its

heavy and light chains are grafted on the framework and constant regions of a human antibody.

14. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is a human antibody.

15. Monoclonal antibody according to anyone of claims 1 to 11, characterized in that it is an IgG1 type antibody.

16. Hybridoma cell, characterized in that it produces monoclonal antibodies according to claims 1 to 13.

17. Composition having antagonist properties to the type I-IFN, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 16.

18. Pharmaceutical composition, characterized in that it comprises monoclonal antibodies according to anyone of claims 1 to 17, together with an appropriate pharmaceutical vehicle.

19. Use of a monoclonal antibody according to anyone of claims 1 to 17, for the manufacture of a drug for the treatment or prophylaxis of a pathological state associated with proliferative cell activity and/or viral cell infection.

20. Process for the selection of a monoclonal antibody having the capacity to recognize the extracellular domain of the human IFN-R and capable of inhibiting the binding of the human type I-IFN, to the IFN-R, characterized by the following steps :

- preincubating a determined concentration of purified monoclonal antibodies according to anyone of claims 1 to 15 or a hybridoma culture supernatant containing monoclonal antibodies, with human cells susceptible of harboring IFN-R ;
- adding labelled human type I-IFN in a determined concentration, to the above preincubating medium ;

- incubating the medium containing the human cells, monoclonal antibodies and labelled type I-IFN for a time sufficient to allow an equilibrium to occur, between the monoclonal antibodies on the one hand and the type I-IFN on the other hand, with the cellular IFN-R ;
- washing the cells ;
- determining the formation of a binding complex between the human cells and the type I-IFN, by counting the amount of attached labelled type I-IFN.

21. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiproliferative activities of the type I-IFN, on human cells characterized by the steps of :

- allowing cells to grow in the presence of human type I-IFN and in the presence of a determined concentration of monoclonal antibodies according to anyone of claims 1 to 15 ;
- counting the cells in order to detect an inhibition of the antiproliferative effect of the type I-IFN.

22. Process for the selection of a monoclonal antibody having the capacity to recognize the extra-cellular domain of the human IFN-R and having a neutralizing capacity against the antiviral activities of the natural, non pathological or pathological type I-IFN on human cells, characterized by the steps of :

- incubating cells with type I-IFN and monoclonal antibodies according to anyone of claims 1 to 15, in determined concentrations, for a time sufficient to allow the formation of a complex

between the monoclonal antibodies and the IFN-R of the human cells and/or between the type I-IFN and the IFN-R of the human cells ;

- infecting the above incubated cells with a determined concentration of a virus ;
- washing the cells ;
- resuspending the cells in culture medium ;
- incubating for a time sufficient to allow the replication of the virus ;
- lysing the cells and ;
- measuring the virus replication or measuring the inhibition of the cytopathic effect.

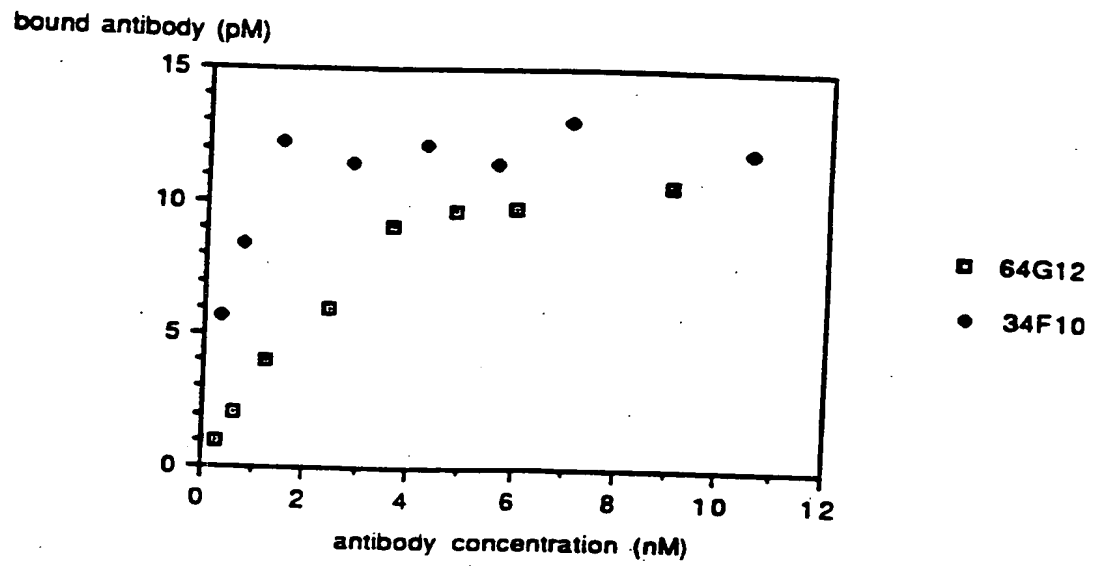
A B R I D G M E N TMONOCLONAL ANTIBODIES AGAINST THE INTERFERON
RECEPTOR, WITH NEUTRALIZING ACTIVITY AGAINST
TYPE I INTERFERON

The invention relates to a monoclonal antibody directed against the human interferon class I receptor (IFN-R) characterized by the following properties :

- it recognizes the extracellular domain of the human IFN-R, and
- it has a neutralizing capacity against the biological properties of the human type I-IFN.

It further concerns their use for the diagnosis.

A



B

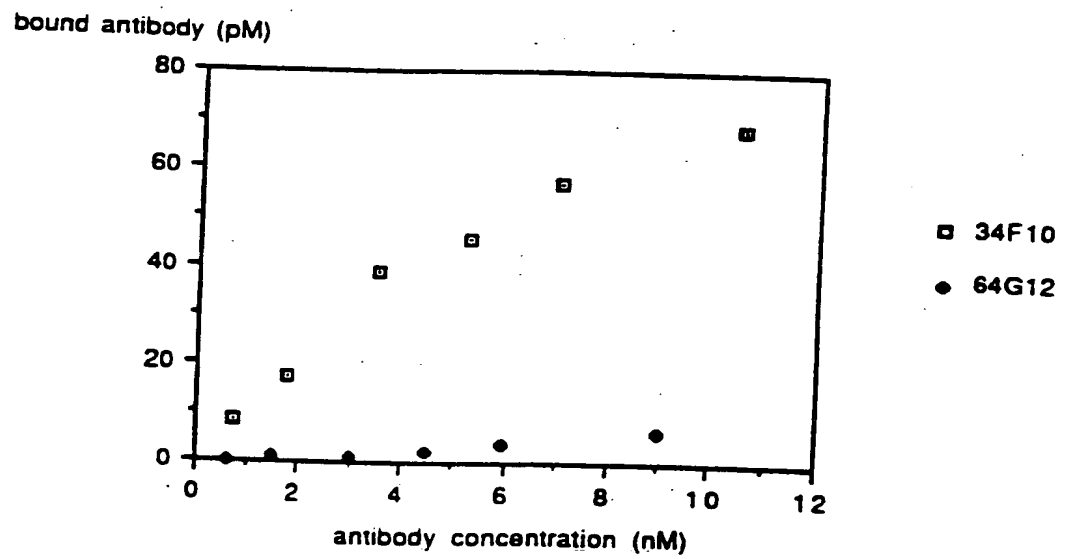


FIGURE 1

CTGCAGGGATCTGCGCGGCTCCCAG

ATG ATG GTC GTC CTC CTG GGC GCG ACG ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 2A

GCA AAC ATG ACC TTT CAA GTT CAG TGG CTC CAC GCC TTT TTA AAA AGG AAT CCT
Ala Asn MET Thr Phe Gln Val Gln Trp Leu His Ala Phe Leu Lys Arg Asn Pro

GGA AAC CAT TTG TAT AAA TGG AAA CAA ATA CCT GAC TGT GAA AAT GTC AAA ACT
Gly Asn His Leu Tyr Lys Trp Lys Gln Ile Pro Asp Cys Glu Asn Val Lys Thr

ACC CAG TGT GTC TTT CCT CAA AAC GTT TTC CAA AAA GGA ATT TAC CTT CTC CGC
Thr Gln Cys Val Phe Pro Gln Asn Val Phe Gln Lys Gly Ile Tyr Leu Leu Arg

GTA CAA GCA TCT GAT GGA AAT AAC ACA TCT TTT TGG TCT GAA GAG ATA AAG TTT
Val Gln Ala Ser Asp Gly Asn Asn Thr Ser Phe Trp Ser Glu Glu Ile Lys Phe

GAT ACT GAA ATA CAA GCT TTC CTA CTT CCT CCA GTC TTT AAC ATT AGA TCC CTT
Asp Thr Glu Ile Gln Ala Phe Leu Leu Pro Pro Val Phe Asn Ile Arg Ser Leu

AGT GAT TCA TTC CAT ATC TAT ATC GGT GCT CCA AAA CAG TCT GGA AAC ACG CCT
Ser Asp Ser Phe His Ile Tyr Ile Gly Ala Pro Lys Gln Ser Gly Asn Thr Pro

GTG ATC CAG GAT TAT CCA CTG ATT TAT GAA ATT ATT TTT TGG GAA AAC ACT TCA
Val Ile Gln Asp Tyr Pro Leu Ile Tyr Glu Ile Ile Phe Trp Glu Asn Thr Ser

AAT GCT GAG AGA AAA ATT ATC GAG AAA AAA ACT GAT GTT ACA GTT CCT AAT TTG
Asn Ala Glu Arg Lys Ile Ile Glu Lys Lys Thr Asp Val Thr Val Pro Asn Leu

AAA CCA CTG ACT GTA TAT TGT GTG AAA GCC AGA GCA CAC ACC ATG GAT GAA AAG
Lys Pro Leu Thr Val Tyr Cys Val Lys Ala Arg Ala His Thr MET Asp Glu Lys

CTG AAT AAA AGC AGT GTT TTT AGT GAC GCT GTA TGT GAG AAA ACA AAA CCA GGA
Leu Asn Lys Ser Ser Val Phe Ser Asp Ala Val Cys Glu Lys Thr Lys Pro Gly

AAT ACC TCT AAA TGAGGTACC
Asn Thr Ser Lys

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FIGURE 2B

CTGCAGGGATCTCCGGCCGCTCCAC

ATG ATG GTC GTC CTC CTG GGC GCG ACC ACC CTA GTG CTC GTC GCC GTG GGC CCA
 MET MET Val Val Leu Leu Gly Ala Thr Thr Leu Val Leu Val Ala Val Gly Pro

TGG GTG TTG TCC GCA GCC GCA GGT GGA AAA AAT CTA AAA TCT CCT CAA AAA GTA
 Trp Val Leu Ser Ala Ala Ala Gly Gly Lys Asn Leu Lys Ser Pro Gln Lys Val

GAG GTC GAC ATC ATA GAT GAC AAC TTT ATC CTG AGG TGG AAC AGG AGC GAT GAG
 Glu Val Asp Ile Ile Asp Asp Asn Phe Ile Leu Arg Trp Asn Arg Ser Asp Glu

TCT GTC GGG AAT GTG ACT TTT TCA TTC GAT TAT CAA AAA ACT GGG ATG GAT AAT
 Ser Val Gly Asn Val Thr Phe Ser Phe Asp Tyr Gln Lys Thr Gly MET Asp Asn

TGG ATA AAA TTG TCT GGG TGT CAG AAT ATT ACT AGT ACC AAA TGC AAC TTT TCT
 Trp Ile Lys Leu Ser Gly Cys Gln Asn Ile Thr Ser Thr Lys Cys Asn Phe Ser

TCA CTC AAG CTG AAT GTT TAT GAA GAA ATT AAA TTG CGT ATA AGA GCA GAA AAA
 Ser Leu Lys Leu Asn Val Tyr Glu Glu Ile Lys Leu Arg Ile Arg Ala Glu Lys

GAA AAC ACT TCT TCA TGG TAT GAG GTT GAC TCA TTT ACA CCA TTT CGC AAA GCT
 Glu Asn Thr Ser Ser Trp Tyr Glu Val Asp Ser Phe Thr Pro Phe Arg Lys Ala

CAG ATT GGT CCT CCA GAA GTA CAT TTA GAA GCT GAA GAT AAG GCA ATA GTG ATA
 Gln Ile Gly Pro Pro Glu Val His Leu Glu Ala Glu Asp Lys Ala Ile Val Ile

CAC ATC TCT CCT GGA ACA AAA GAT AGT GTT ATG TGG GCT TTG GAT GGT TTA AGC
 His Ile Ser Pro Gly Thr Lys Asp Ser Val MET Trp Ala Leu Asp Gly Leu Ser

TTT ACA TAT AGC TTA CTT ATC TGG AAA AAC TCT TCA GGT GTA GAA GAA AGG ATT
 Phe Thr Tyr Ser Leu Leu Ile Trp Lys Asn Ser Ser Gly Val Glu Glu Arg Ile

GAA AAT ATT TAT TCC AGA CAT AAA ATT TAT AAA CTC TCA CCA GAG ACT ACT TAT
 Glu Asn Ile Tyr Ser Arg His Lys Ile Tyr Lys Leu Ser Pro Glu Thr Thr Tyr

TGT CTA AAA GTT AAA GCA GCA CTA CTT ACG TCA TGG AAA ATT GGT GTC TAT AGT
 Cys Leu Lys Val Lys Ala Ala Leu Leu Thr Ser Trp Lys Ile Gly Val Tyr Ser

CCA GTA CAT TGT ATA AAG ACC ACA GTT GAA AAT GAA CTA CCT CCA CCA GAA AAT
 Pro Val His Cys Ile Lys Thr Thr Val Glu Asn Glu Leu Pro Pro Pro Glu Asn

ATA GAA GTC AGT GTC CAA AAT CAG AAC TAT GTT CTT AAA TGG GAT TAT ACA TAT
 Ile Glu Val Ser Val Gln Asn Gln Asn Tyr Val Leu Lys Trp Asp Tyr Thr Tyr

FIGURE 3A

ATT GAA AAT ATA AGC ACA ATT GCT ACA GTA GAA GAA ACT AAT CAA ACT GAT GAA
 Ile Glu Asn Ile Ser Thr Ile Ala Thr Val Glu Glu Thr Asn Gln Thr Asp Glu

GAT CAT AAA AAA TAC AGT TCC CAA ACT AGC CAA GAT TCA GGA AAT TAT TCT AAT
 Asp His Lys Lys Tyr Ser Ser Gln Thr Ser Gln Asp Ser Gly Asn Tyr Ser Asn

GAA GAT GAA AGC GAA AGT AAA ACA AGT GAA GAA CTA CAG CAG GAC TTT GTA TGA
 Glu Asp Glu Ser Glu Ser Lys Thr Ser Glu Glu Leu Gln Gln Asp Phe Val

CCAGAAATGAACTGTGTCAAGTATAAGGTTTTTCAGCAGGAGTTACACTGGTACC

↑
 1697

FIGURE 3C

*Revoir la demande
d'invention*

IN THE CLAIMS:

Please delete claims 1-22 and insert the following new claims:

- 23. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 27 to amino acid residue 427 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12 (deposited at the ECACC under no. 92022605). --
24. A peptide or polypeptide as claimed in claim 23, consisting of amino acid residue 27 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof.
25. A peptide or polypeptide which is a fragment of the extracellular portion of the IFN-R of SEQ ID NO: 2, said peptide or polypeptide consisting of amino acid residue 1 to amino acid residue 229 of SEQ ID NO: 1 or 2 or a portion thereof; wherein said peptide or polypeptide specifically binds to monoclonal antibody 64G12.
26. An analogue of a peptide or polypeptide as claimed in claim 23, which is derived from said peptide or polypeptide by substitution of one or more amino acid residues and which retains the ability to specifically bind to monoclonal antibody 64G12.
27. A method of producing a monoclonal antibody, comprising immunizing an animal with a peptide or polypeptide as claimed in claim 23, fusing spleen cell from the immunized animal with myeloma cells, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide.
28. A method of producing a monoclonal antibody, comprising contacting stimulated B-lymphocytes *in vitro* with a peptide or polypeptide according to claim 23, fusing the resultant B-lymphocytes with B-lymphocytes immortalized with Epstein-Barr

virus, isolating hybridoma cells which produce antibodies, and selecting and purifying monoclonal cell lines producing antibodies which specifically bind to said peptide or polypeptide. --

IN THE ABSTRACT

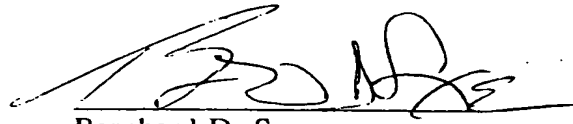
Please insert the Abstract provided on the attached sheet.

REMARKS

The Examiner is respectfully requested to enter the above amendments prior to examination of the instant application. Support for the amendments is present throughout the specification, in particular at pages 10-11.

Respectfully submitted,

February 2, 1999
Date


Bernhard D. Saxe
Reg. No. 28,665

FOLEY & LARDNER
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Suite 500
Washington, D.C. 20007-5109
Tel: (202) 672-5300

ABSTRACT OF THE DISCLOSURE

A monoclonal antibody is provided which is directed against the human interferon type I receptor (IFN-R), which recognizes the extracellular domain of the human IFN-R and which has neutralizing capacity against the biological properties of human type I-IFN. Diagnostic and therapeutic applications for the monoclonal antibody also are provided.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name;

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

MONOCLONAL ANTIBODIES AGAINST THE INTERFERON RECEPTOR,
WITH NEUTRALIZING ACTIVITY AGAINST TYPE I INTERFERON

(Attorney Docket No. 017283/0123)

the specification of which (check one)

☐ Is attached hereto.

☒ Was filed on March 30, 1993 as Application Serial No. PCT/EP93/00770 and was amended on _____ (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe that the same invention was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Cod of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claimed?	Certified Copy Attached?
92400902.0	European	31/March/1992	Yes	NO

I HEREBY CLAIM the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Application Number	Parent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of FOLEY & LARDNER:

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ALAN I. CANTOR	Reg. No. 28,163
WILLIAM T. ELLIS	Reg. No. 26,874
JOHN J. FELDHAUS	R g. No. 28,822
MICHAEL D. KAMINSKI	Reg. No. 32,904
LYLE K. KIMMS	R g. N . 34,079

KENNETH E. KROSIN	R g. No.	25,735
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GLENN LAW	Reg. No.	34,371
PETER G. MACK	Reg. No.	26,001
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SYBIL MELOY	Reg. No.	22,749
RICHARD C. PEET	Reg. No.	35,792
GEORGE E. QUILLIN	Reg. No.	32,792
ANDREW E. RAWLINS	Reg. No.	34,702
BERNHARD D. SAXE	Reg. No.	28,665
CHARLES F. SCHILL	Reg. No.	27,590
RICHARD L. SCHWAAB	Reg. No.	25,479
MICHELE M. SIMKIN	Reg. No.	34,717
HAROLD C. WEGNER	Reg. No.	25,258

to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith.

I request that all correspondence be directed to:

Bernhard D. Saxe
Foley & Lardner
At Washington Harbour
3000 K Street, NW, Suite 500
Washington, DC 2000

Telephone: 202-672-5472
Facsimile: 202-672-5399

I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

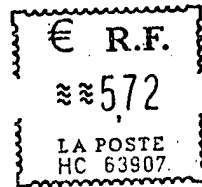
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Residence	<i>Paris, France</i>
Citizenship	<i>FRANCE</i>
Post Office Address	<i>24, rue Jonquoy, F-75014 Paris, France</i>
Inventor's signature	
Date	

Name of second inventor	<i>Francois MEYER</i>
Residence	<i>Paris, France</i>
Citizenship	<i>LUXEMBOURG</i>
Post Office Address	<i>3 Place du Panthéon, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of third inventor	<i>Deborah MAGUIRE</i>
Residence	<i>Paris, France</i>
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Post Office Address	<i>24, rue Maître-Albert, F-75005 Paris, France</i>
Inventor's signature	
Date	
Name of fourth inventor	<i>Ivan PLAVEC</i>
Residence	<i>Sunnyvale, CA, USA</i>
Citizenship	<i>CROATIA</i>
Post Office Address	<i>1415, Mallard Way, Sunnyvale, CA 94087, USA</i>
Inventor's signature	
Date	
Name of fifth inventor	<i>Michael G. TOVEY</i>
Residence	<i>Paris, France</i>
Citizenship	<i>GREAT BRITAIN</i>
Post Office Address	<i>7, rue Lagrange, F-75005 Paris, France</i>
Inventor's signature	
Date	

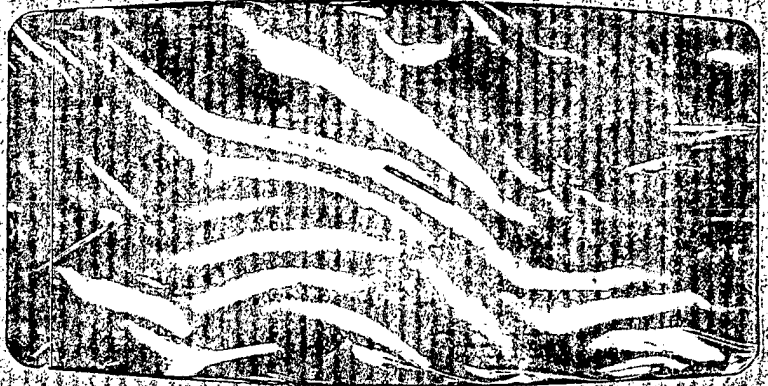


Ernest GUTMANN
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SA

3, rue Chauveau-Lagorie
75008 PARIS



jus / VMA / A /
1608 ACA



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RECOMMANDE

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29 OCT. 2001

E. GUTMANN - Y. PLASSERAUD S.A.

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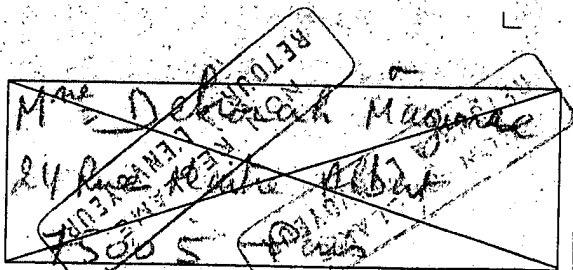
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DE VOTRE ENVOI
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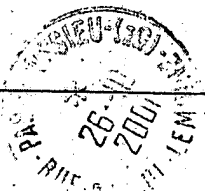
Présenté le :

Distribué le
Signature du destinataire :



RETOUR A :

gutmann. Plasseraud
3 rue Chauveau Lagorie
75008 Paris



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Annex 20

